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Field of CHEMISTRY

DEVELOPMENT OF MULTIFUNCTIONAL MATERIALS BASED ON CHITOSAN IMINE DERIVATIVES

Summary of the doctoral thesis

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We inform you that on November 7, 2025, at 10:00, in the Conference Hall of the "Petru Poni" Institute of Macromolecular Chemistry in Iași, the public defense of the doctoral thesis entitled "Development of multifunctional materials based on chitosan imine derivatives" by Ramona Socea (married Lungu), will take place, to achieve the scientific title of doctor.

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In accordance with the Regulation on the organization and conduct of the doctoral program for the award of scientific titles in the Romanian Academy, we are sending you the summary of the doctoral thesis with the request to communicate your appreciations and observations. On this occasion, we invite you to participate in the public defense of the doctoral thesis.

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INTRODUCTION

Contemporary society is marked by an increasingly intense global concern for environmental protection and human health, factors that require a reconsideration of the materials used in industry, biomedicine, and other related fields. In this regard, the development of sustainable and multifunctional polymeric materials has become a strategic priority at the international level.

Although synthetic polymers have revolutionized many industries, from medicine, automotive, and aerospace to electronics, packaging, and food, they are largely non-degradable and contribute to environmental pollution. These limitations led to a reorientation of scientific research towards identifying environmentally friendly alternatives, particularly **biopolymers**, that offer functional performance similar or superior to that of conventional polymers. From this perspective, **the research topic** proposed in this thesis is motivated by the urgent need to identify alternative solutions based on biopolymers with functional performance comparable to or superior to that of synthetic polymers.

Chitosan, a derivative of chitin, stands out as one of the most promising compounds due to its complex functional profile: biodegradability, biocompatibility, lack of toxicity, antimicrobial, antifungal, hemostatic, immunomodulatory, and antioxidant activity. These properties make it suitable for various applications in the biomedical, biotechnological, and food industries, as well as in the production of environmentally friendly packaging. The growing interest in chitosan is confirmed by numerous scientific publications, international projects, and continuously expanding industrial applications. Historically, chitosan was first obtained in 1859 by Charles Marie Benjamin Rouget by treating chitin with potassium hydroxide, resulting in an acid-soluble derivative, initially called "chitine modifiée". The term "chitosan" was formalized in 1894 by Felix Hoppe-Seyler. Although its potential was recognized as early as the first decades of the 20th century, its development was slowed down by the dominance of synthetic polymers. Starting in the 1970s, awareness of their negative impact on the environment led to a reassessment and exploitation of the unique properties of chitosan.

With the increase in demand for environmentally friendly materials, there has been a growing interest in **multifunctional materials** capable of performing multiple functions simultaneously. In this context, the **chemical modification of chitosan** at reactive hydroxyl and amine groups has become an effective strategy for improving its performance. The reaction with various aldehydes or ketones leads to the **formation of Schiff bases (dynamic imine bonds)**, generating smart materials capable of responding to external stimuli such as pH, temperature, or light.

Among the materials based on **imine derivatives of chitosan**, the most studied are: (i) **hydrogels**, which can retain large amounts of water and maintain their structural stability, making them ideal for applications such as tissue regeneration, controlled drug delivery, or wound healing; (ii) **nanofibers**, obtained by techniques such as electrospinning, which offer a large specific surface area and adjustable porosity, essential qualities in dressings, biosensors, filtration membranes, or water purification; (iii) **functional coatings**, especially edible ones, used in the food industry as sustainable alternatives to synthetic packaging, providing protection against microbial contamination and prolonging the freshness of perishable products. Thus, **materials**

based on imine derivatives of chitosan, due to their structural and functional versatility, have remarkable potential in regenerative medicine, biotechnology, agriculture, and the food industry, supporting the global transition to sustainable, efficient, and environmentally friendly technologies.

Within this scientific and technological context, the doctoral thesis entitled ***"Development of multifunctional materials based on imine derivatives of chitosan"*** is part of a current interdisciplinary and strategic research direction, both internationally and nationally.

The research hypothesis starts from the premise that the chemical modification of chitosan through **imination reactions with bioactive aldehydes** can lead to the development of **smart, dynamic, and multifunctional materials** capable of responding to external stimuli and ensuring the controlled release of active substances, with applications in wound healing and active food packaging**.**

Based on this hypothesis, the **scientific objectives** of the paper were as follows: **O1** - *Obtaining and characterizing supramolecular hydrogels based on imine derivatives of chitosan, with potential in wound treatment*; **O2** - *Developing functionalized nanofibers for medical applications, with a focus on antimicrobial activity and biocompatibility*; **O3** - *Formulating functional antimicrobial coatings for cellulose paper-based packaging intended for the food industry*.

The thesis is organized into **two main parts** and comprises five chapters. **Part I** contains **Chapter 1**, which provides a brief overview of the literature on chitosan, its chemical modifications, as well as the main methods of synthesis, properties, and applications of the most representative chitosan-based materials and their imine derivatives. **Part II** presents the results of the author's own research and includes **Chapters 2, 3, 4,** and **Chapter 5**, which details the materials, synthesis and characterization methods, equipment used, and additional experimental data.

Chapter 1 is structured into five subchapters, each dealing with important aspects of chitosan and its derivatives. *Subchapter 1* provides an overview of chitosan, focusing on its physicochemical properties highlighted in recent literature data, as well as the main strategies used for its structural modification. *Subchapter 2* explores the modification of chitosan through imine reactions, presenting general elements of imine chemistry, the reversible nature of imine bonds, and ways to exploit their dynamic behavior. *Subchapters 3, 4,* and *5* review the literature on methods for obtaining chitosan-based hydrogels, nanofibers, and coatings, emphasizing their properties and potential applications in various fields.

Chapter 2 is dedicated to the preparation and characterization of dynamic supramolecular hydrogels based on iminochitosan and is structured in two subchapters: (i) the development of a hydrogel obtained by crosslinking chitosan with boronic aldehyde, evaluated for its potential in wound treatment; (ii) the development of chitosan-based hybrid hydrogels, in which two bioactive agents are incorporated through reversible imine bonds, targeting applications in wound healing. The first subchapter has the specific objective of evaluating the enzymatic degradation of the hydrogel depending on pH, simulating the variable composition of exudate during the wound healing process. *The second subchapter* explores a new approach in chitosan-based hydrogel chemistry: the crosslinking of chitosan with two bioactive aldehydes and the impact on the hydrogelation mechanism, morphology, and functionality of the obtained hydrogels.

Both the boronic aldehyde-crosslinked hydrogel and the hybrid hydrogels, obtained by using an equimolar mixture of two aldehydes: salicylaldehyde, 4-hydroxybenzaldehyde, vanillin, piperonal, citral, and boronic aldehyde, were characterized structurally, supramolecularly, and morphologically. In order to validate their potential for medical application, enzymatic degradation in media with variable pH was evaluated to correlate the stability of the hydrogels with the physiological stages of wound healing, antimicrobial and antioxidant activity, as well as biocompatibility demonstrated by *in vitro* tests. In addition, the reversibility of the imine bond, its correlation with the gradual release of bioactive aldehydes, and its influence on the functionality of the hydrogels were evaluated.

Chapter 3 presents the preparation and characterization of new multifunctional nanofiber materials based on imine derivatives of chitosan, with improved functional properties. For this purpose, chitosan nanofibers were functionalized by condensation reaction using a mixture of two bioactive aldehydes, boronic aldehyde and citral, in varying proportions. The obtained materials were characterized in terms of composition and morphology, and the forces between the fibers' components, which play an essential role in their properties, were evaluated. Properties relevant to their application as biodegradable wound dressings were also investigated: swelling capacity, antimicrobial activity, aldehydes' release kinetics, and *in vitro* biocompatibility.

Chapter 4 presents the development of new citryl-imino-chitosan-based formulations with antimicrobial activity, intended to be used as functional coatings for cellulose paper packaging. The formulations were synthesized by the imination reaction between chitosan and citral, a compound chosen for its antimicrobial and hydrophobic properties, which can improve the water resistance of the cellulose substrate. The materials obtained were characterized structurally, morphologically, and thermally using specific analytical methods. Particular attention was paid to investigating the impact of paper coating on changes in mechanical and barrier properties, followed by an evaluation of the formulations' effectiveness in extending the shelf life of food products by performing relevant functional tests on model fruit.

Chapter 5 presents the experimental part, which includes the methods for preparing hydrogels, fibers, and coatings based on imine derivatives of chitosan, as well as the materials, equipment, and techniques used in conducting the studies. It also includes spectra, figures, and tables with additional data that support and supplement the results obtained and presented in chapters 2, 3, and 4.

The proposed doctoral thesis represents a significant and timely contribution to the field of materials science and biomedicine through the exploration and development of **multifunctional materials** with remarkable properties in the form of **hydrogels**, **nanofibers**, and **coatings**. The novelty and importance of this work lie in its unique approach, which combines three key elements:

Firstly, the use of chitosan as the base polymer. As a biocompatible and biodegradable biopolymer, **chitosan** is an excellent starting point for the design of materials for medical applications, minimizing the risk of rejection and promoting natural integration into tissues. This strategic choice underscores the thesis's focus on safe and effective solutions for health.

Secondly, the major innovation lies in the use of **biologically active aldehydes** in the structure of the synthesized materials. The aldehydes selected in the studies presented in the thesis are not chosen randomly; they have essential properties, such as **antimicrobial** and/or **antioxidant activity, while also being biocompatible**. This dual approach, used in Chapters 2

and 3, is vital in the clinical context, where infections and oxidative stress are major factors that can compromise the healing process. In Chapter 4, citral was chosen for its hydrophobic nature, which contributes to improving the water resistance of the cellulose support. By integrating these active agents, the resulting systems take on an expanded therapeutic role, going beyond their simple physical support function.

Thirdly, the defining element of this thesis is the formation of reversible imine bonds between chitosan and aldehydes. This dynamic chemistry not only allows for the creation of smart and adaptable materials, but also enables the controlled and sustained release of aldehydes at the site of action. The reversibility of the imine bonds also contributes to the controlled biodegradation of the material, avoiding the need for additional removal interventions. This feature positions the developed systems as advanced solutions with significant potential in applications such as wound healing, where targeted release of therapeutic agents and safe degradation are essential.

Thus, this doctoral thesis contributes significantly not only to expanding fundamental knowledge in chemistry and materials engineering but also opens new horizons for the development of personalized and effective therapeutic solutions with a direct and positive impact on human health.

The research results were disseminated through scientific articles and conference presentations, and were financially supported by national and international research projects, reflecting the impact and relevance of the topic addressed.

Chapter 2. Imino-chitosan-based hydrogels for biomedical applications

2.1. Synthesis and characterization of a new hydrogel based on chitosan and 2-formylphenylboronic acid

2.1.1. Introduction

Extensive wounds caused by conditions such as burns or diabetes are a major health problem because slow healing increases the risk of infection and serious complications [1–3]. Therefore, researchers are looking for solutions for rapid wound closure, including the development of polymeric hydrogels, particularly those based on polysaccharides, which are non-toxic, biodegradable, and biocompatible [4–7]. Chitosan stands out among these polysaccharides, accelerating wound healing by stimulating hemostasis, cell proliferation, and collagen deposition [8,9]. According to numerous studies, chitosan-based materials, especially those with antibacterial agents, can prevent infections [10]. Since removing traditional dressings can damage new tissue, studies are focusing on creating biodegradable dressings that resorb as the wound heals [11]. Therefore, chitosan is a promising candidate in this regard. This study investigated the biodegradation rate of a chitosan-based hydrogel (crosslinked with 2-formylphenylboronic acid) as a function of wound exudate pH. The aim was to create a biodegradable, biocompatible, and antimicrobial biomaterial for wound healing by monitoring mass loss and observing the samples by SEM microscopy at different pH levels corresponding to the healing stages.

2.1.2. Results and discussions

2.1.2.1. Structural and supramolecular characterization

The H2 hydrogel was obtained by the acid condensation reaction of chitosan (Ch) with 2-formylphenylboronic acid, hereinafter referred to as boronic aldehyde (2-FPBA) (Figure 2.1.). The experimental protocol for obtaining the hydrogel presented in the literature was modified by performing the synthesis in water, avoiding the use of other organic solvents. The formation of the hydrogel was visually validated by the inverted tube test (Figure 2.4.c).

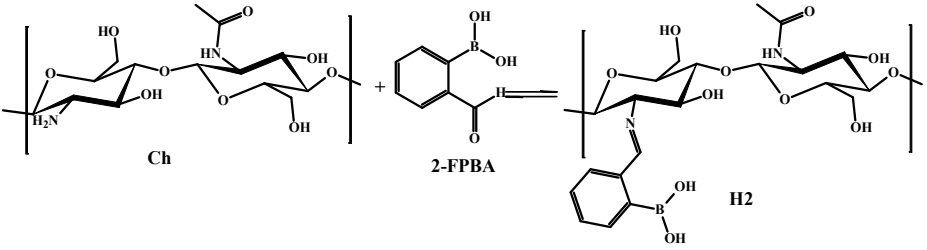


Figure 2.1. The reaction of chitosan with boronic aldehyde

Structural analysis (^{1}H-NMR and FTIR) confirmed the formation of imine bonds in the hydrogel, indicating a reversible imination equilibrium influenced by the aqueous reaction environment. 1H-NMR investigations performed at different time intervals showed an increase in the degree of conversion of aldehyde to imine bonds, from 18.9% at the initial moment to 26.1% after 24 hours, subsequently stabilizing at this value.

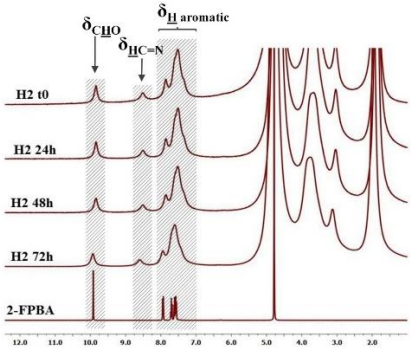


Figure 2.2. ^{1}H-NMR spectra of the hydrogel and 2-FPBA aldehyde

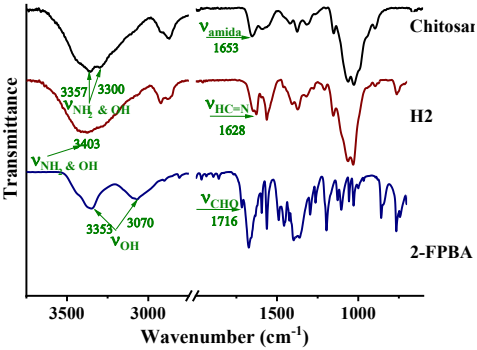
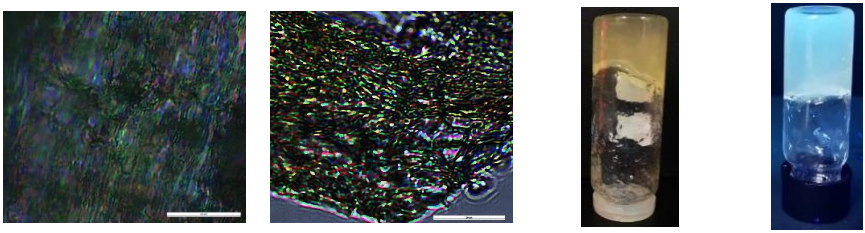


Figure 2.3. FTIR spectra of chitosan, 2-FPBA, and freeze-dried hydrogel

On the other hand, in the FTIR spectrum of the lyophilized hydrogel, the appearance of the absorption band characteristic of the imine bond at 1628 cm-1 and the absence of the absorption band of the aldehyde group [12] could be observed, proving that the equilibrium of the imination reaction was shifted towards the products during the lyophilization process, along with the removal of water (Figure 2.3.).

The POM images of the hydrogel showed light birefringence with a striped texture due to the self-assembly of imine units formed during hydrogelation into clusters that act as crosslinking nodes (Figure 2.4. a,b) [13]. In addition, the hydrogel exhibited blue light emission when illuminated with a UV lamp, consistent with the formation of supramolecular fluorophores (Figure 2.4.d) [14].



a) **b)** **c)** **d)**

Figure 2.4. Images of the hydrogel in polarized light (scale: 20 µm), taken on the thick (a) and thin (b) hydrogel samples, and images of the hydrogel in normal light (c) and under UV light (d).

2.1.2.2. *In vitro* biological properties

The cell viability of normal human dermal fibroblasts was tested to assess the cytotoxicity of the hydrogel, in accordance with ISO 10993-5:2009(E). The results showed that, at concentrations of 2-FPBA below 0.284%, the hydrogel is not cytotoxic and has a cell viability of

over 70% (**Figure 2.5.**), indicating that the systems can be safely used for bioapplications that require contact with fibroblast cells [15].

The evaluation of the antimicrobial properties of **H2** hydrogel showed that it has strong antibacterial activity against relevant pathogens, reaching inhibition zones of 9 mm (*S. Aureus*), 15 mm (*E.coli*), and 17 mm (*C. albicans*) [16]. The antimicrobial activity was correlated with the reversibility of the imine bond, which favored the shift of the equilibrium towards the reactants once they were consumed in the process of destroying the pathogens.

2.1.2.3. *In vitro* biodegradation assessment

A study was conducted on the biodegradation of the hydrogel in environments with different pH values, similar to the pH values of exudate during the wound healing period.

The results obtained in this study indicate that the hydrogel is biodegradable in the presence of biological agents, such as human serum, and that the degradation process occurs in a controlled manner, with the formation of monomers and oligomers. The hydrogel degraded rapidly in an environment similar to that specific to wounds. Firstly, the mass loss in the presence of lysozyme is significantly higher than in its absence (45% *vs.* 17%), clearly indicating the effect of lysozyme in the degradation of chitosan (**Figure 2.6.**). In addition, it can be seen that the pH of the lysozyme solution influenced the rate of biodegradation. Thus, at alkaline pH values (8.5 and 9), specific to wounds in the initial healing phase, biodegradation is slightly accelerated and allows for faster release of the antimicrobial agent.

At pH 10, specific to the later stages of healing, the rate decreased slightly, while at pH 5.5, specific to normal skin, the hydrogel degraded very rapidly, being completely absorbed on the first day. The evolution of degradation was supported by SEM images, which indicated a porous morphology in basic pH environments and its transformation into a fibrous one in acidic pH environments (5.5), suitable for tissue regeneration.

These data suggest that the hydrogel has the potential to be rapidly adsorbed by newly formed tissue without the need for traumatic debridement. This could promote trauma-free tissue regeneration and thus scar-free wound healing.

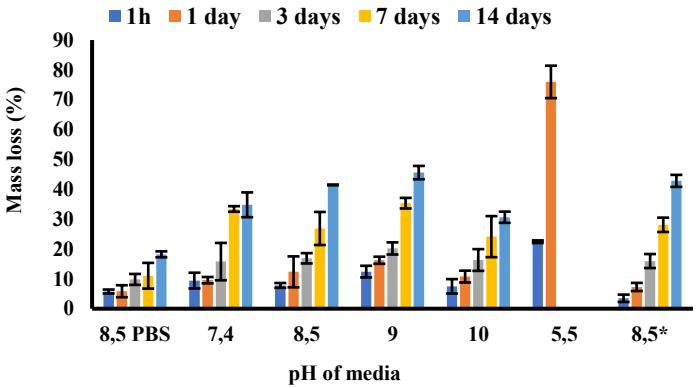


Figure 2.6. Mass loss of the hydrogel over 14 days

2.2. Crosslinking of chitosan with mixtures of bioactive monoaldehydes

2.2.1. Introduction

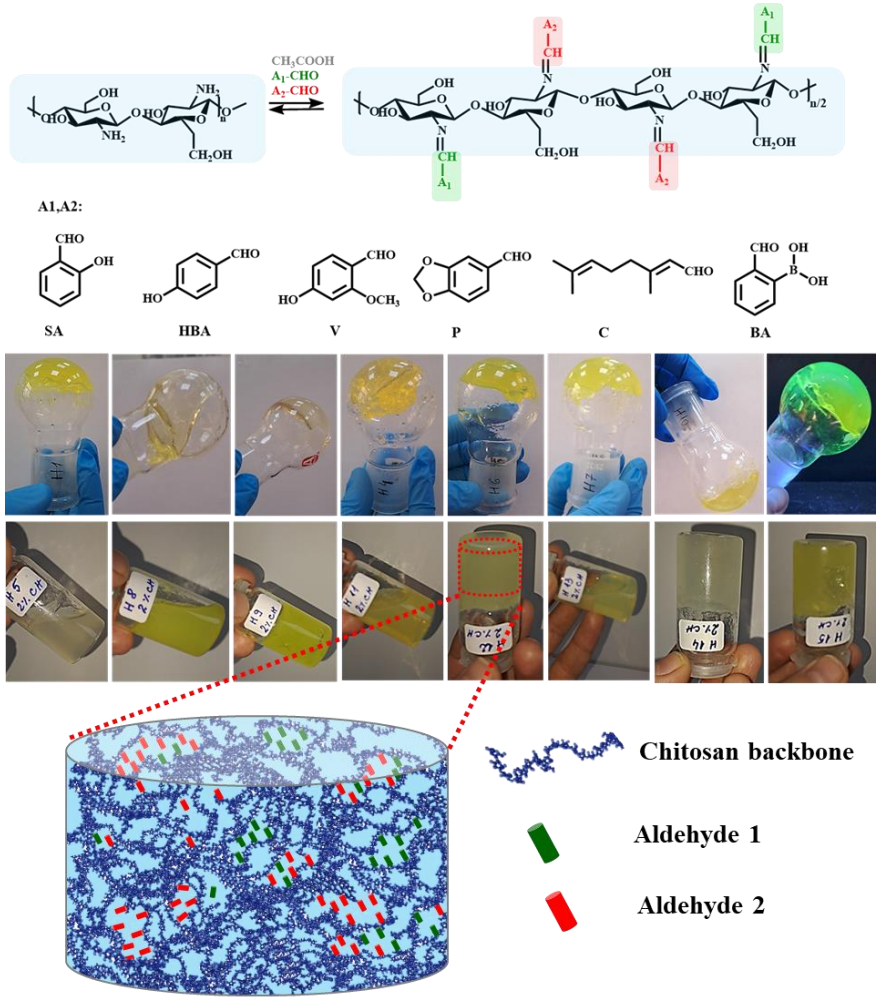
Chitosan-based hydrogels are essential in biomedicine, although traditional crosslinking with dialdehydes presents toxicity issues [17]. Recent studies have developed an innovative method for cross-linking chitosan with monofunctional aldehydes, which are less toxic and may also exhibit bioactivity. This involves the formation of reversible imine bonds and their self-assembly into hydrophobic clusters, giving hydrogels controllable properties such as antimicrobial activity, chiral character, and self-healing [18]. To obtain multifunctional biomaterials, the study presented in this subchapter proposes the crosslinking of chitosan with mixtures of bioactive aldehydes. The objective is to develop hydrogels for wound healing by encapsulating agents with antimicrobial and/or antioxidant effects, exploiting their synergism and the dynamic nature of imine bonds for controlled release and biodegradation [19].

2.2.2. Results and discussions

A series of 15 hybrid hydrogels was synthesized by condensation reaction of chitosan with binary mixtures of 6 bioactive aldehydes: salicylaldehyde (SA), p-hydroxybenzaldehyde (PHB), vanillin (V), piperonal (P), citral (C), and boronic aldehyde (BA) (**Table 2.1.** and **Scheme 2.1.**). In addition, six control hydrogels were obtained, each with a single aldehyde, to facilitate a clearer interpretation of the results. Most hydrogels were obtained at a chitosan concentration of 2% (Table 2.1.- black color). However, those containing vanillin and p-hydroxybenzaldehyde required a higher concentration of 3% to hydrogel (Table 2.1. blue color).

Table 2.1. Composition of hybrid hydrogels and control hydrogels

Sample	Aldehyde(s)	Sample	Aldehydes	Sample	Aldehydes
H-SA	SA	**H2**	PHB /V	**H9**	SA/BA
H-BA	BA	**H3**	PHB /P	**H10**	V/P
H-P	P	**H4**	PHB /C	**H11**	V/C
H-C	C	**H5**	PHB /BA	**H12**	V/BA
H-V	V	**H6**	SA/V	**H13**	P/C
H-PHB	PHB	**H7**	SA/P	**H14**	P/BA
H1	PHB/SA	**H8**	SA/C	**H15**	C/BA



Scheme 2.1. Reaction for obtaining chitosan-based hybrid hydrogels. Images of the obtained hydrogels and graphical representation of their supramolecular architecture

To understand the mechanism of hydrogel formation, they were investigated by FTIR, ^{1}H-NMR, and UV-Vis spectroscopy, X-ray diffraction (WXRD), and polarized optical microscopy (POM).

The FTIR spectra of all hydrogels showed intense absorption bands around 1630 cm^{-1}, characteristic for imine bond vibration. Compared to the control hydrogels, the hybrid hydrogels showed a slight variation in the position of this band, attributed to the overlap of the two bands associated with the two imines formed. When two aldehydes forming imines with distinct bands in the control hydrogels were used, for example C (1646 cm^{-1}) *vs.* BA (1627 cm^{-1}) [20], their

position was maintained in the hybrid hydrogels, confirming that both aldehydes acted as cross-linking agents.

The analysis of ^{1}H-NMR spectra (**Figure 2.12.**) highlighted the dynamic equilibrium between aldehyde and imine in the chitosan gelation processes, with the ratio of imine/aldehyde proton integrals (I_I/I_A) and the degree of imination being used as comparative parameters. In 2% solutions, the systems showed progressive increases in the I_I/I_A ratio, suggesting the strengthening of hydrogel networks, while others showed decreases, indicating predominantly physical gelation. The stability of imine bonds depends on the structural characteristics of aldehydes (π-π segregation, hydrophobicity, stabilization by H bonds). Thus, SA, BA, and C showed high conversions (up to 47%), unlike V, P, and PHB (≈5%). In hybrid systems, simultaneous ignition led to higher conversion rates than the sum of the individual ones, an effect favored by increased viscosity. In the case of aldehydes with antagonistic reactivity, the degree of ignition was determined by the most reactive one, confirming the role of viscosity in shifting the equilibrium towards the products.

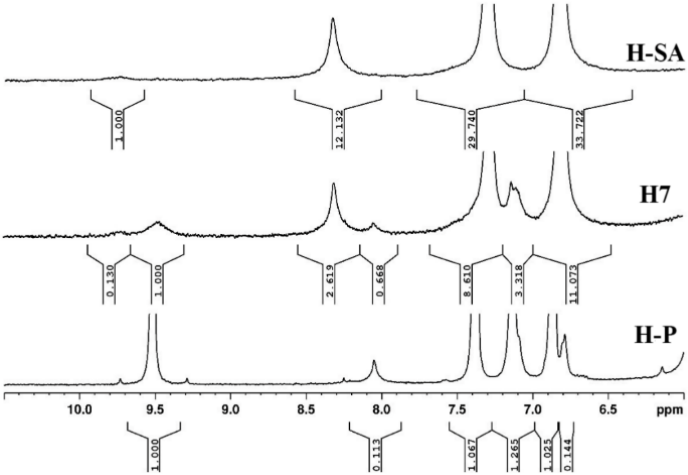
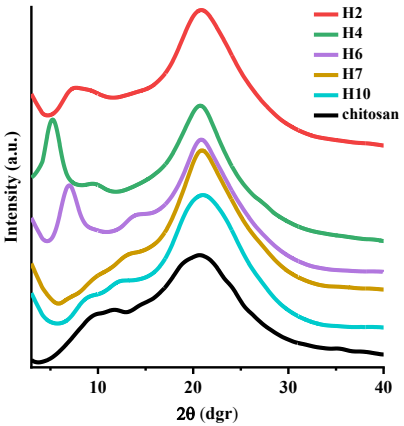


Figure 2.12. ^{1}H-NMR spectrum of hybrid hydrogel **H7** (SA/P) compared to control hydrogels

2.2.2.3. Supramolecular characterization by WXRD and POM

X-ray diffraction (XRD) revealed the presence of reflections at small angles, indicating an ordered structure with large distances between layers. Although some contribution of both aldehydes is observed in the mixtures, most evidence support the hypothesis that crosslinking occurs predominantly through the formation of homogeneous clusters, generally from imines of the same type. Polarized optical microscopy (POM) revealed strong birefringence, similar to that observed in pure hydrogels (**Figure 2.13.**).



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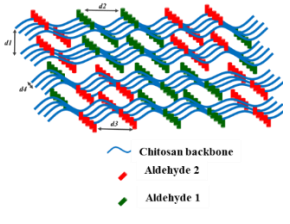

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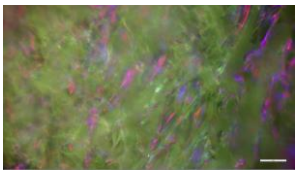


e)

Figure 2.13. a) X-ray diffraction patterns obtained on xerogels, **d**) schematic representation of an ordered portion of the supramolecular architecture of xerogels, and **e)** POM image of **H8** xerogel (SA/C) (scale: 20 µm)

2.2.2.4. Morphological study by SEM

The morphology of hydrogels is essential for their functional properties (hydration rate, swelling, mucoadhesiveness, and diffusion of various bioactive principles). The study showed that while xerogels obtained from BA-based reference hydrogels have a well-defined and interconnected porous network, those containing PHB and V have fragmented morphology and perforated walls. This aspect was also highlighted in the case of hybrid hydrogels. It was also observed that the pore size in hybrid hydrogels is smaller than that of reference hydrogels.

2.2.2.5. Study of the viscoelastic behavior of hydrogels

Rheological analysis confirmed that most samples formed stable hydrogels with an elastic modulus higher than the plastic modulus. The exception is **H2** (V, PHB), which according to ^{1}H-NMR has the lowest degree of imination and therefore could not generate a three-dimensional network. It was found that the degree of imination influenced the viscoelastic properties, with the most elastic hydrogels being obtained at a medium degree of imination. Furthermore, crosslinking agents played an important role in the gelation process.

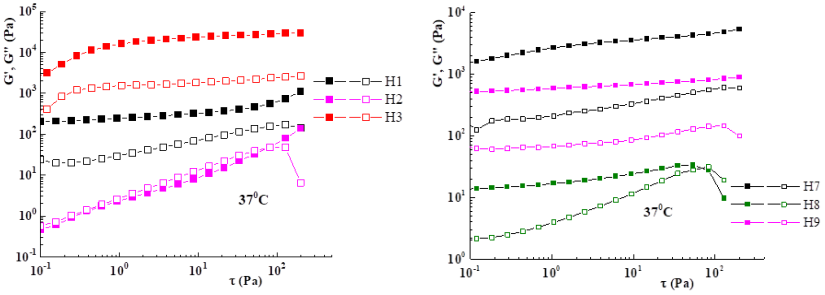


Figure 2.15. Rheological parameters for representative hybrid hydrogels

For example, salicylaldehyde (SA) produced highly elastic hydrogels, as in the case of hydrogels **H1** (PHB/SA) and **H7** (SA/P), while boronaldehyde (BA) reduced elasticity, suggesting a contribution of physical forces in crosslinking, such as **H9** (SA/BA) (**Figure 2.15**).

Biological functionality

2.2.2.6. *In vitro* release studies

The release could be monitored in the case of hydrogels containing aldehydes whose absorption bands did not overlap in UV-Vis. All tested hydrogels showed rapid aldehyde release in the first 8 hours, a phenomenon that was more pronounced in samples **H12** (V/BA) and **H14** (P/BA) and less evident in sample **H9** (SA/BA). This behavior is determined by the degree of imination values: a lower degree of imination resulted in a faster release and a higher percentage of aldehyde released.

2.2.2.7. Enzymatic degradation studies

Hybrid hydrogels underwent rapid degradation from an initial 50% for H14 (P/BA) to 85% after 21 days for H8 (SA/C). This acceleration is linked to the instability of mixed imine clusters and increased water accessibility. Furthermore, the degree of imination determined the rate of degradation: reduced cross-linking favors greater mass losses, e.g., H14 (P/BA), H15 (C/BA), while a high degree of imination stabilizes the network, e.g., H10 (V/P). In the SEM images after degradation, porous structures and tangled fibers similar to the semi-interpenetrated network model can be observed, as in the case of samples **H8** (SA/C) and **H15** (C/BA) (**Figure 2.17.**).

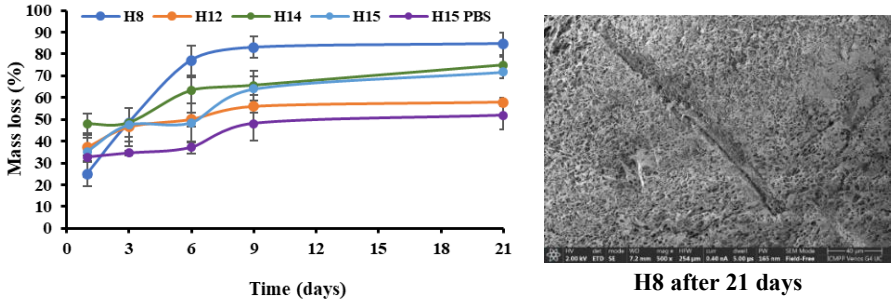


Figure 2.17. Mass loss following enzymatic degradation and SEM micrographs of H8 xerogel following the biodegradation process

2.2.2.8. Antimicrobial activity

Most hybrid hydrogels demonstrated effective antimicrobial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria, and *C. albicans* fungus. The best antifungal results were obtained for **H14** (BA/P), with inhibition zones of up to 48.84 mm against *C. albicans* and 34 mm against *S. aureus*. In the case of the Gram-negative strain *E. coli*, the **H9** hydrogel showed the best activity, with an inhibition zone of up to 35 mm (**Figure 2.18.**).

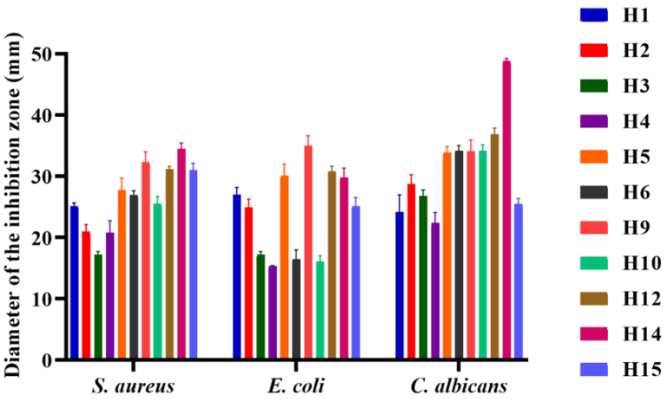


Figure 2.18. Antimicrobial activity of the analyzed samples

2.2.2.9. Antioxidant activity

The evaluation of antioxidant activity using the DPPH test showed that all monoaldehydes, especially vanillin, have the ability to inhibit this free radical. No clear synergism was observed in the aldehyde mixtures, but neither was any mutual inhibition of activity.

The hydrogels exhibited much higher antioxidant activity than the individual monoaldehydes, due to the contribution of chitosan (**Figure 2.19**). For H7 (SA/P), H9 (BA/SA), H13 (P/C), and H14 (BA/P), a synergistic effect between chitosan and aldehydes was even observed. The data obtained indicate that hydrogels have a high potential for inhibiting free radicals, which is useful in biomedical applications where reducing oxidative stress is beneficial, such as biomaterials for wound healing.

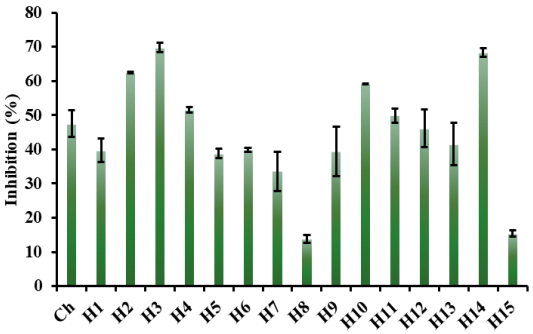


Figure 2.19. Antioxidant activity against DPPH of hydrogels compared to chitosan

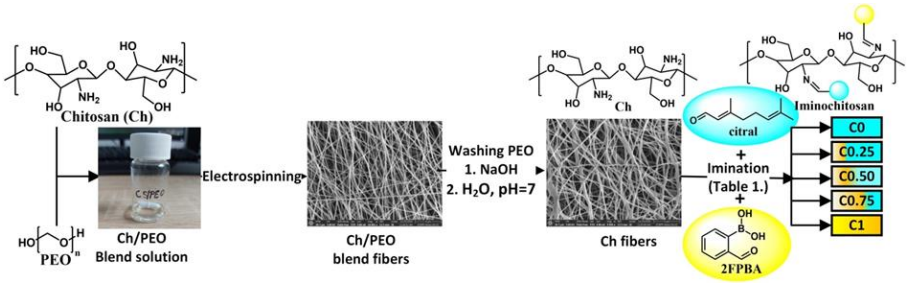
3.1. Introduction

The treatment of wounds, especially chronic wounds and burns, is a major public health problem due to slow healing and an increased risk of invasive infections [21]. Chitosan-based nanofibers have shown promise due to their favorable properties: biocompatibility, porosity, antimicrobial and hemostatic activity [22,23]. To improve the multifunctionality of chitosan-based nanofibers in order to increase wound healing capacity, solutions should be identified to control the release rate of the antimicrobial compound and its bioavailability at the site of infection. In this context, the following study proposes the functionalization of chitosan nanofibers through imine bonds with two bioactive aldehydes (2-formylphenylboronic acid and citral) to achieve a synergistic and controlled antimicrobial effect. This innovative approach, previously unexplored, could lead to the development of effective wound healing materials.

3.2. Results and discussions

3.2.1. Synthesis

The functionalized fibers were prepared in three stages: obtaining chitosan/polyethylene oxide (Ch/PEO) fibers by electrospinning, removing PEO to obtain pure chitosan fibers, and functionalizing them by imination reaction with mixtures of boronic aldehyde and citral in various molar ratios. The samples were labeled according to the boronic aldehyde fraction (**Scheme 3.1.**).



Scheme 3.1. Schematic representation of the preparation of functionalized chitosan fibers and their codes

FTIR and ^{1}H-NMR analyses on chitosan fibers confirmed that both aldehydes, citral and boronic aldehyde, interacted with the amine groups. It was observed that the degree of conversion of the amine groups into imine bonds was maximum at 56% when only boronic aldehyde was used and decreased with increasing proportion of citral, most likely due to the lower reactivity of the latter.

3.2.3. The dynamic character of imine units

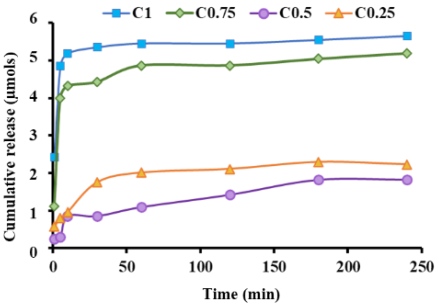


Figure 3.5. Comparison of the amounts of boronic aldehyde released over time for samples **C1**, **C0.75, C0.5, C0.25**

Monitoring the release of aldehydes in PBS saline solution by UV-Vis indicated a faster release of 2-FPBA compared to citral. This can be explained by the hydrophilic nature of 2-FPBA, which favored the shift of the imination equilibrium towards the reagents, while the hydrophobic nature of citral prevented the release. Comparing the amounts of 2-FPBA released from the samples containing it, a similar trend to that of the degree of imination was observed, higher amounts of 2-FPBA were released from samples with higher degrees of imination. Thus, the amount of 2-FPBA released in 240 minutes corresponded to that grafted onto chitosan by imination (**Figure 3.5.**).

3.2.4. Morphology

SEM microscopy showed that the unfunctionalized fibers have a smooth morphology and well-defined porosity. Functionalization with citral resulted in rough, interconnected fibers, while 2-FPBA generated smoother fibers with a smaller diameter. Functionalization with both aldehydes generates fibers with intermediate roughness and diameters. The different behavior was attributed to the hydrophilic/hydrophobic nature of the two aldehydes. In polarized light, chitosan fibers exhibit birefringence, a sign of the alignment of chitosan chains during electrospinning and the ordered phase. This is maintained in fibers with 2-FPBA, but is diminished in those with citral, probably due to the coverage with aliphatic chains.

3.2.5. Swelling behavior

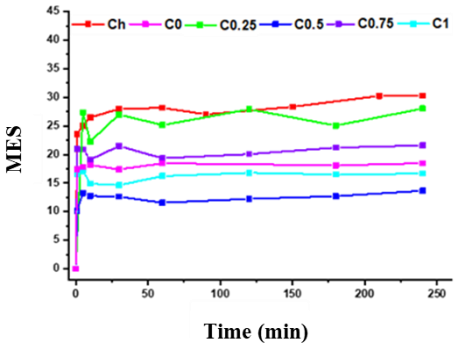


Figure 3.9. Swelling kinetics of the fibers

For in vivo application, fiber swelling in PBS at 37 °C was evaluated. Chitosan fibers had a higher swelling degree than functionalized fibers, confirming the decrease in porosity through predominantly surface functionalization. All samples initially swelled rapidly, then had a slight decrease in MES, followed by stabilization, indicating shape retention and favoring the transport of bioactive agents. Sample **C0.25** showed an alternating increase/decrease in MES values, possibly correlated with an alternating removal of the two aldehydes, a behavior of interest for drug delivery medical devices

(**Figure 3.9. a**). The main conclusion of the swelling behavior study is that all fibers are capable of rapid swelling, having the potential to adsorb wound exudate without significantly changing their shape and thus ensuring good transport of bioactive agents.

3.2.6. Enzymatic biodegradation

The biodegradation of the fibers in a lysozyme environment was monitored for 21 days. After one day, the chitosan fibers lost 35% of their mass, those with boronic aldehyde (**C1**) lost around 20%, and the samples with citral lost less than 15%. Subsequently, degradation was slower, finally reaching around 40% for C1 and less for the citral samples. These findings confirm that hydrophobic citral inhibits the biodegradation process. SEM images showed coalescence and exfoliation of the fibers, indicating predominantly surface biodegradation.

3.2.7. In vitro biocompatibility

The biocompatibility of the newly synthesized biomaterials was tested on normal human dermal fibroblasts (NHDF), and cell viability was evaluated (less than 70% cytotoxic effect). Chitosan fibers showed a cell viability of about 95%, which dropped to 80% for samples containing citril-imine units and to 75% for those containing 2-FPBA-based imine units (**Figure 3.11.**). Samples containing both types of imine maintained cell viability greater than 80%. These data indicated that all synthesized biomaterials meet the biosafety assessment criterion and can be applied as biomedical devices that come into contact with living tissues.

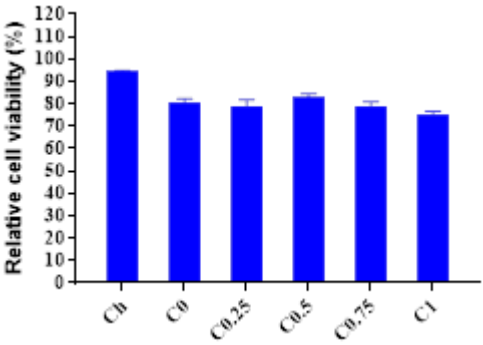


Figure 3.11. Viability of NHDF cells in contact with the studied nanofibers

3.2.8. Antimicrobial properties

Although chitosan is known as an antimicrobial agent, the non-functionalized fibers did not show any inhibition zone, probably due to the low mobility of macromolecules in the solid state. However, fibers functionalized with 2-FPBA (**C0.5**, **C0.75**, **C1**) showed clear inhibition, especially against *A. brasiliensis* (**Figure 3.12.c**). The higher activity of citral-imine fibers suggests a synergistic effect between citral and 2-FPBA, as also indicated by sample C1, for

which an increase in 2-FPBA content did not lead to additional efficacy. All functionalized samples (including those with citral) prevented the adhesion and formation of bacterial and fungal biofilms (*S. aureus*, *E. coli*, *C. albicans*, *C. glabrata*, *A. brasiliensis*), demonstrating their role as a protective barrier for wounds.

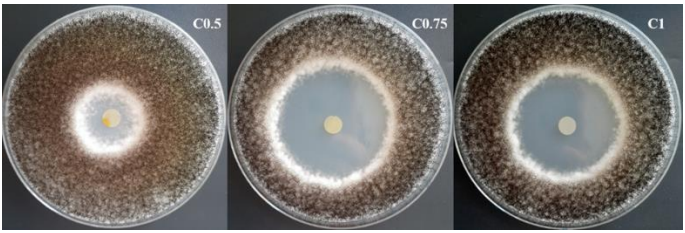
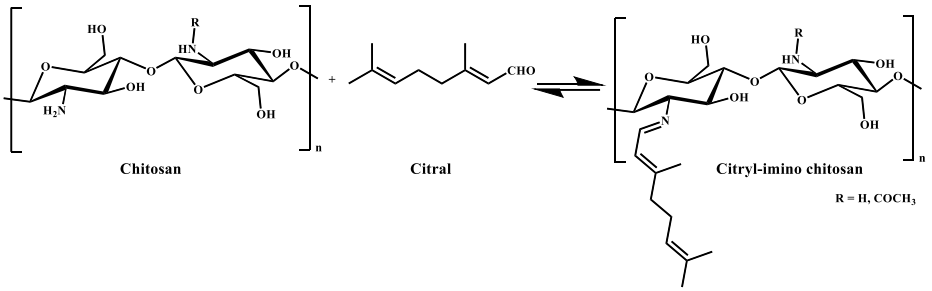


Figure 3.12. c) Antifungal activity of the tested dressings against *A. Brasiliensis*

Due to the environmental problems caused by traditional plastic packaging, cellulose-based materials such as paper represent a sustainable alternative [24]. However, the reduced hydrophobic and barrier properties of paper limit its use, requiring improvements. An effective solution is to coat the paper with biopolymers such as chitosan, a natural, biodegradable material with antimicrobial properties [25]. This study focuses on an environmentally friendly technique for coating paper with a chitosan derivative obtained by reaction with citral, a natural aldehyde with antimicrobial and hydrophobic properties [26]. The aim is to improve the mechanical and barrier properties of the paper, particularly its water resistance, in order to extend the shelf life of food. This creates a multifunctional, environmentally friendly, and effective packaging.

4.2. Results and discussions

The citrime-imine derivatives of chitosan were synthesized prior to application by the acid condensation reaction of chitosan with citral (**Scheme 5.1.**), in different molar ratios of glucosamine/aldehyde units, namely 1/1 (code **C1**) and 2/1 (code **C2**), and the solutions obtained were then applied to paper. The non-functionalized chitosan solution (code **C**) was applied under similar conditions to the imine derivatives.



Scheme 5.1. Synthesis of citryl-imine derivatives of chitosan

Structural analysis confirmed the formation of the imine bond in both citryl-imino-chitosan derivatives and paper coated with each of them by the appearance of an absorption band at 1641 cm^{-1} in FTIR and chemical shifts at 179, 180, 181 ppm in ^{13}C-NMR. Furthermore, the FTIR spectra provided useful information on the potential interactions between the paper surface and chitosan or citryl-imino-chitosan, on the one hand by increasing the density of hydrogen bonds and the crystallinity index in all coated paper samples, and on the other hand by the presence of a new absorption band at 1651 cm^{-1} attributed to the vibration of imine bonds that could form between the aldehyde groups of residual lignin in kraft paper and the amine groups in chitosan.

4.2.3. Thermal properties

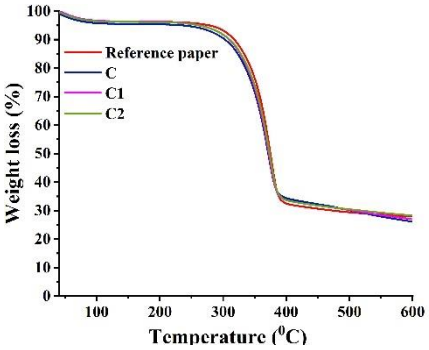


Figure 4.5. TGA curves of the samples

Thermogravimetric analysis (TGA) showed that the reference paper degraded at 375 °C. Coating with chitosan or citryl-imino-chitosan derivative slightly reduced the decomposition temperature due to the lower thermal stability of chitosan. However, the citryl-imino-chitosan derivative provided slightly higher thermal stability than chitosan, strengthening the paper fibers. In addition, the degradation temperature of the coated paper is much higher than that required for the packaging or even sterilization process, indicating its safe use without risk of degradation (**Figure 4.5.**).

4.2.4. Strength properties

Treating paper with chitosan and with the corresponding imine derivatives significantly improves its mechanical properties. This makes the paper more resistant to tensile stress, tearing, and repeated bending, as well as more flexible (**Figure 4.6.**). These improvements are the result of the formation of new bonds between the coating polymers and the cellulose fibers. Coating with citryl-imino-chitosan solution has proven to be more effective due to its lower viscosity and the crosslinking ability of citral, which facilitates better integration into the paper structure.

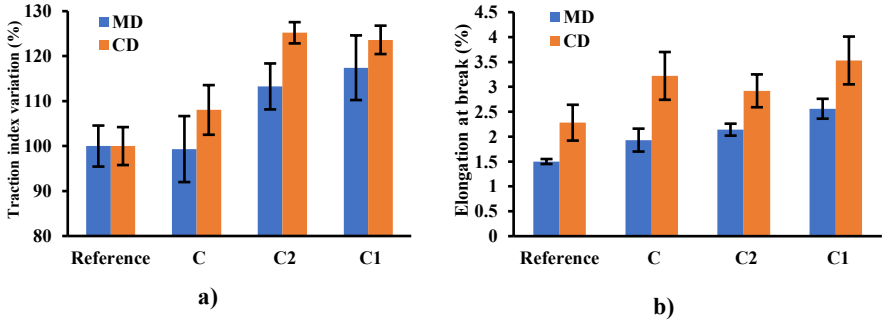


Figure 4.6. a) variation in the traction index (TI) and **b)** elongation at break of coated paper samples compared to reference paper

4.2.5. Barrier properties

Coating paper with citril-imino-chitosan significantly improves its water and gas barrier properties. This reduces water adsorption (Cobb value) (Figure 4.8. a) and increases hydrophobicity (contact angle), especially on the more porous side of the paper, by filling the

pores. In addition, the coating creates an effective barrier that decreases the water vapor transmission rate, helping to maintain the freshness of packaged products. Although the low viscosity of the solution allows deeper penetration than chitosan and a slight increase in air permeability (**Figure 4.9.a**), the barrier capacity of the material remains adequate for packaging.

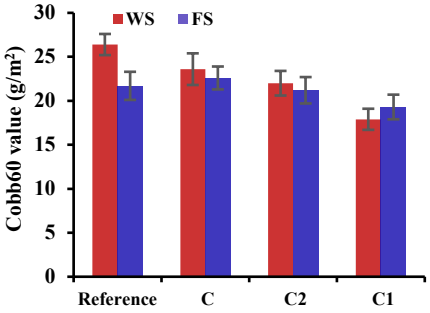


Figure 4.8. a) Water adsorption capacity of the wire side (WS-coated) and felt side (FS) of samples coated with solutions **C, C1, C2**

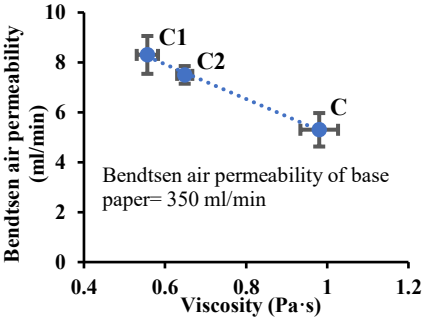
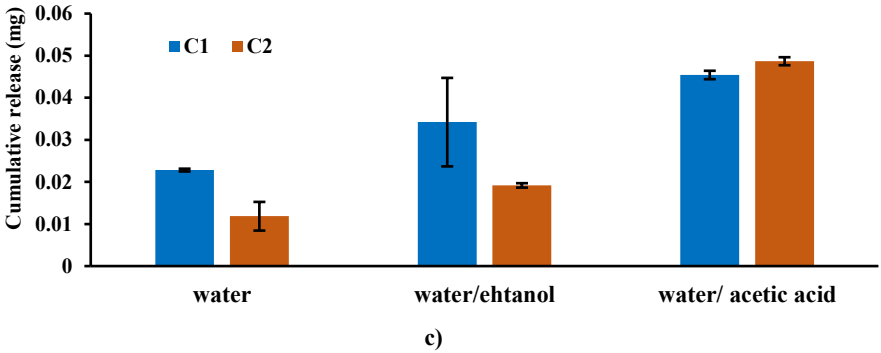


Figure 4.9. a) Relationship between Bendtsen air permeability of coated paper samples and viscosity of coating solutions

4.2.6. Food preservation

4.2.6.1. Release of citral

The release of citral from the coated paper over a period of 24 hours was studied in solutions that reproduce food environments: water, 50% ethanol, and an aqueous solution with pH of 3.84 (characteristic of acidic fruits such as raspberries) (**Figure 4.11.c**). The results showed that the release was the fastest in the acidic environment, due to the shift of the equilibrium towards the reactants, followed by ethanol, where citral has a higher solubility, and finally water. In ethanol and water, the amount of citral released was proportional to the initial amount, while in acidic environment, the opposite trend was observed. In a parallel experiment, with a single sampling at 24 h, no statistically significant differences were recorded between samples, the values being comparable to those obtained cumulatively in the previous experiment. Therefore, the release process is dynamic, influenced by pH and solubility limit, by the properties of the release medium.

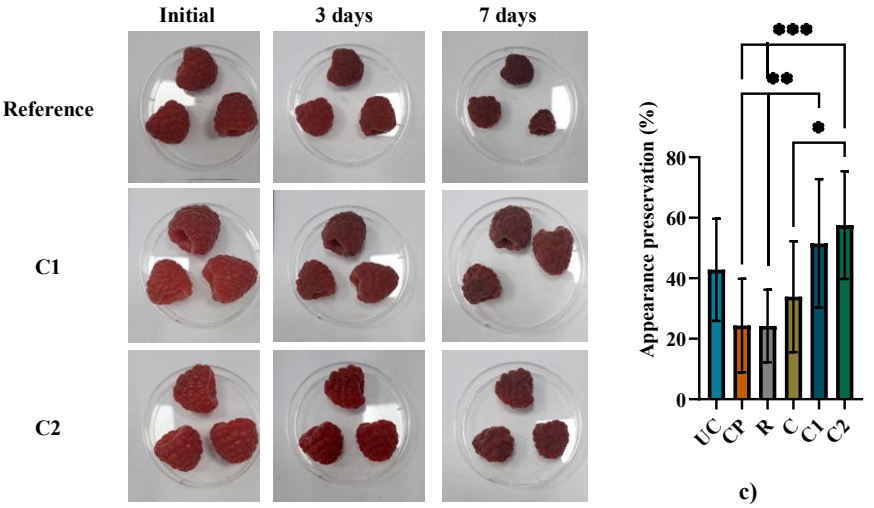


c)

Figure 4.11. Graphical representation of the cumulative amount of citral c) released from C1 and C2

4.2.6.2. Visual assessment and hedonic test

To assess the potential of coated paper to extend the shelf life of food, raspberries were selected as the model food due to their perishability and susceptibility to mold contamination. During the 7 days of the experiment, better preservation of raspberries covered with citryl-imino-chitosan impregnated paper was visually observed. This observation was also supported by the results of the hedonic test, which showed higher scores for all five criteria: appearance, color, aroma, texture, and overall acceptability (**Figure 4.12.**).



c)

Figure 4.12. Representative images of the fruit during the 7 days of the experiment and **c)** Graphical representation of the preservation of overall acceptability (UC: uncovered; CP: commercial packaging; R: reference) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

4.2.6.3. Color

The color difference index (ΔE) values indicated that the covered fruits had a much slower color degradation (ΔE ~6.5) compared to the uncovered control group (ΔE ~11), confirming the protective effect of the coating. Although all samples showed color degradation over time, the coatings significantly slowed down this process.

4.2.6.4. Firmness

Compared to commercial packaging, fruits covered with impregnated paper showed better firmness values. The best results were obtained for the fruits covered with sample **C2**, which required an average force of 6 N to be compressed. The difference was not statistically significant compared to fruit covered with commercial packaging, which required a force of 4 N (**Figure 4.13.**).

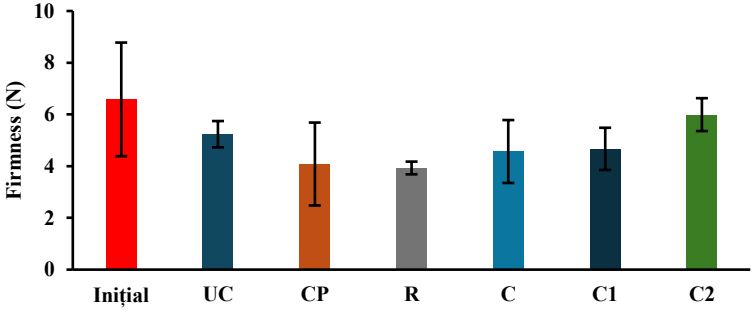


Figure 4.13. Representation of fruit firmness (UC: uncovered; CP: commercial packaging; R: reference)

4.2.6.5. Antimicrobial properties

To consume fruit safely, it is essential that it is free from microorganisms. The chitosan film packaging created an effective barrier against pathogens, and the addition of citral doubled this inhibitory effect (**Table 4.5.** and **Figure 4.14.**).

Table 4.5. Average values of viable mesophilic bacteria and yeast/mold (log CFU·g^{-1}) in raspberries after 7 days of incubation

Samples	Bacteria	Yeasts/Molds
Uncovered	6.45 ± 0.03	5.91 ± 0.02
Commercial packaging	6.06 ± 0.04	5.76 ± 0.04
Reference	6.30 ± 0.03	5.85 ± 0.02
C	5.76 ± 0.01	5.80 ± 0.02
C1	5.02 ± 0.03	5.35 ± 0.04
C2	4.74 ± 0.04	4.84 ± 0.04

This effect was also visible in the case of yeasts and molds, where the number of viable strains decreased significantly in sample C2 compared to the commercial packaging, demonstrating superior protection.

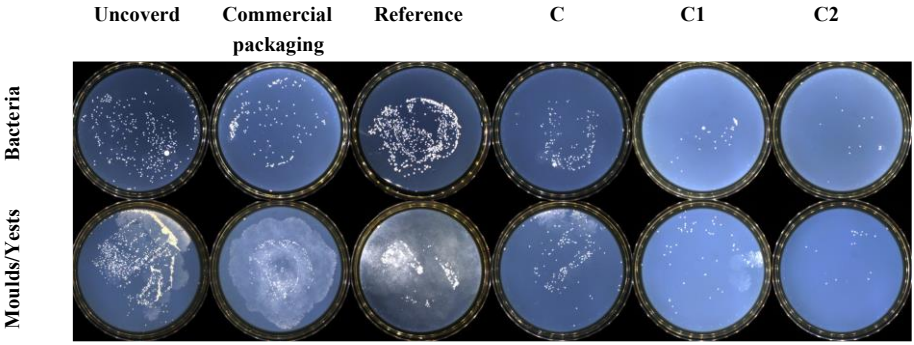


Figure 4.14. Microbiological load of raspberries after 7 days of storage

The doctoral thesis entitled "**Development of multifunctional materials based on chitosan imine derivatives**" has **178** pages divided into **5** chapters that include **14** tables, **56** figures, **7** diagrams, and **401** bibliographic notes. The thesis is structured in two parts: a literature review, which is presented in Chapter 1, and personal contributions (Chapters 2–5). The thesis concludes with a series of general conclusions.

The original results are presented in three chapters and cover:

• Synthesis and characterization of new imino-chitosan-based hydrogels for biomedical applications

• Obtaining and characterizing a series of nanofibers functionalized with citral and 2-formylphenylboronic acid

• Synthesis and characterization of new eco-friendlys food packaging based on imino-chitosan derivatives

Based on the studies conducted, the following general conclusions were drawn:

Chapter 2. Imino-chitosan-based hydrogels for biomedical applications

- **Synthesis and characterization of a new hydrogel based on chitosan and 2-formylphenylboronic acid**

- A hydrogel was obtained by a procedure involving the acid condensation reaction of the amino groups of chitosan (Ch) with the formyl group of 2-formylphenylboronic acid (2-FPBA) in water. The hydrogel state was confirmed by the inverted tube test.

- The ^{1}H-NMR spectra indicated the establishment of an imine equilibrium during hydrogelation, with the presence of chemical shifts specific to imine and aldehyde protons. The degree of conversion of aldehyde to imine increased from 18.9% in the first minutes to 26.1% after one hour, consistent with the shift of the imination equilibrium towards products during hydrogelation.

- The FTIR spectrum of the lyophilized hydrogel confirmed the appearance of the absorption band characteristic of the imine bond at 1628 cm^{-1}, demonstrating the success of the imination reaction. Spectral changes were observed in the hydrogen bond region, consistent with the formation of intermolecular interactions between 2-FPBA and chitosan, as well as intramolecular interactions formed by the imine derivative of chitosan.

- Polarized light microscopy images indicated strong birefringence with a banded texture, characteristic of layered supramolecular architectures formed by hydrophobic/hydrophilic segregation of imine units into ordered clusters acting as crosslinking nodes.

- *In vitro* biocompatibility assessment of the hydrogel on normal human dermal fibroblasts (NHDF) revealed cell viability higher than 70% for a 2-FPBA content of less than 0.284%, indicating that the hydrogel can be safely used as a biomedical device.

- The hydrogel demonstrated strong antimicrobial activity, inhibiting relevant pathogens: *S. Aureus, E. coli*, and *C. Albicans*, at concentrations of 2-FPBA at which the hydrogel is cytocompatible. Thus, at a concentration of 0.142% 2-FPBA, the hydrogel significantly reduced the *C. albicans* load within 24 hours (99.99%), this efficacy being attributed to the reversibility of the imine bond, which allows continuous action against pathogens.

- The evaluation of enzymatic degradation under conditions that mimic the evolution of wound exudate pH during healing process highlighted the hydrogel's potential to function as a resorbable ointment, thus avoiding traumatic debridement and supporting scar-free tissue regeneration.

- It has been observed that at physiological pH (7.4), the mass loss of hydrogel is moderate (32%), but increases to 45% at the alkaline pH (8.5-9) specific to wound exudate in the first hours after its production, promoting faster biodegradation and the release of antimicrobial aldehyde for protection against infections. Degradation increases slightly by increasing the lysozyme concentration to a level characteristic of the exudate from infected tissues and accelerates at acidic pH, specific to the skin (pH=5.5), with total biodegradation of the hydrogel being achieved in two days.

- Investigation of the morphology of the hydrogel at different stages of biodegradation confirmed the drastic influence of the presence of lysozyme and the pH of the environment: *(i)* at high pH (9.5–10), the hydrogel undergoes marked degradation but retains a porous structure, (ii) at low pH (5.5), massive degradation leads to a fibrous structure, ideal for tissue regeneration, while (iii) in the absence of the enzyme, the pores collapse, reflecting partial degradation and limited aldehyde release.

➢ **Crosslinking chitosan with mixtures of bioactive monoaldehydes**

- A series of 15 hybrid hydrogels were synthesized by acid condensation reaction between chitosan and an equimolar mixture of two aldehydes (salicylaldehyde, 4-hydroxybenzaldehyde, vanillin, piperonal, boronic aldehyde, and citral), for a 2:1 molar ratio between glucosamine and formyl units. Under similar conditions, control hydrogels were obtained by reacting chitosan with monoaldehydes.

- Preliminary confirmation of hydrogels' formation was performed using the inverted tube test, which allowed for the re-evaluation of working protocols for cases where gelation did not occur. Specifically, in cases where 4-hydroxybenzaldehyde and vanillin and mixtures were used, the chitosan concentration was increased from 2% to 3% to obtain soft solids indicating hydrogel formation.

- FTIR spectra confirmed the formation of imine bonds by the presence of specific bands in the vicinity of 1630 cm^{-1}. In the case of hybrid hydrogels, the overlap of the specific bands of the two imine groups was observed, confirming the contribution of both aldehydes to the hydrogelation

process. Changes were also observed in the spectral regions corresponding to the amine and hydroxyl groups, reflecting changes in the supramolecular structure.

- ^{1}H-NMR analysis confirmed the dynamic nature of the hydrogels, due to the reversibility of imine bond formation. Hydrogels with 2% chitosan showed an increased imine/aldehyde ratio and degree of imination, indicative of a consolidated chemical network, while hydrogels with 3% chitosan showed a low imine/aldehyde ratio and degree of imination, indicative of predominantly physical gelation. Hybrid systems demonstrated a synergistic effect in shifting the imination equilibrium towards products.

- X-ray diffraction revealed the appearance of a small-angle reflection, corresponding to the periodicity of large distances, characteristic of the stratified self-organization of imine units on chitosan chains in ordered clusters. In the diffractograms of hybrid hydrogels, these reflections appear at values intermediate to those in the reference hydrogels, suggesting a common assembly of imines, dominated by the aldehyde with the highest reactivity and stability.

- The morphology of the control xerogels revealed a porous network with a fragmented architecture in the case of those generated by 4-hydroxybenzaldehyde and vanillin. In comparison, the hybrid hydrogels exhibited a distinct porous morphology with voids in the pore wall structure, a morphological feature suggesting the formation of a secondary network, similar to the formation of interpenetrating networks.

- The hydrogel state was confirmed by rheological analysis, which showed higher elastic than plastic modulus values for most of the systems studied. Rheological studies also highlighted the influence of the degree of crosslinking on viscoelastic properties, indicating that the chemical nature and physical interactions of co-crosslinked aldehydes play a decisive role in establishing the gel state and modulating thixotropic behavior.

- The release of aldehydes from hydrogels, monitored by UV-Vis analysis, showed a rapid initial release in the first 8 hours, depending on the degree of imination: a more pronounced release for systems with a low degree of imination, consistent with the presence of a large amount of free aldehyde, and a slower release for hydrogels with a high degree of imination.

- Hybrid hydrogels showed accelerated degradation compared to reference hydrogels, with a mass loss of up to 85% in 21 days. This behavior was correlated with the formation of ordered hybrid clusters with structural defects, which were more susceptible to liquid infiltration.

- The hydrogels exhibited antioxidant activity, with a free radical inhibition capacity of up to 70%, and an improvement proportional to the activity of the cross-linking aldehydes.

- Most hydrogels exhibited remarkable antimicrobial properties, correlated with the activity of the constituent aldehydes. Thus, systems obtained from aldehydes with complementary activity led to hydrogels with a broad spectrum of action.

- The MTS test indicated that, apart from four hydrogels, the systems studied did not affect the viability of fibroblast cells, maintaining cytotoxicity values above 70%, in accordance with the standard applied to biomedical devices. Furthermore, images obtained by optical microscopy confirmed that most cells maintained their elongated shape and morphology without significant

changes, supporting the MTS test data and the high potential for applications involving direct contact with living tissues. However, the lack of a clear correlation between cell viability and the chemical structure or degree of crosslinking of the hydrogels makes it necessary to repeat this investigation.

Chapter 3. Obtaining and characterization of nanofibers functionalized with citral and 2-formylphenylboronic acid

- To obtain multifunctional biomaterials, chitosan fibers were functionalized through a three-step process: electrospinning of Ch/PEO fibers, removal of PEO to obtain pure chitosan fibers, and functionalization of these fibers through imination reaction with a mixture of 2-FPBA and citral in different molar ratios.

- FTIR spectra demonstrated that surface functionalization of the fibers led to the formation of imine units in the case of both aldehydes (citral and 2-FPBA), evidenced by characteristic vibration bands at 1646 cm^{-1} (citral) and 1634 cm^{-1} (2-FPBA). Spectral changes in the 3680-3000 cm^{-1} range indicated changes in the hydrogen bond network through the formation of new bonds, both intermolecular and intramolecular.

- The yield of the imination reaction with 2-FPBA on the fiber surface is influenced by both the reactant concentration and by the presence of citral, which has higher reactivity and leads to inhibition of imination with 2-FPBA by clogging the surface.

- The UV-Vis release study showed a significantly faster release of 2-FPBA compared to citral, due to its hydrophilic nature. The release took place in two stages, and the release profile was closely interdependent with the degree of imination, without being significantly affected by the presence of citral. The results suggest that the imination equilibrium can be shifted towards reactants under the action of external stimuli.

- Polarized light microscopy showed birefringence for all fibers, before and after functionalization, consistent with the alignment of chitosan chains during electrospinning, leading to a degree of crystallinity and, consequently, good mechanical properties.

- All samples showed rapid initial swelling, followed by a slight decrease in swelling at equilibrium corresponding to the release of aldehyde and dissolution of chitosan, and then stabilization at values ranging from 12 to 27 g/g during the 14-day investigation period. This behavior demonstrates, on the one hand, the ability of the fibers to retain liquids, such as wound exudate, and, on the other hand, the possibility of transporting bioactive agents, which are important aspects in the design of wound dressings.

- Enzymatic biodegradation showed an inverse trend with hydrophobic citral content: citryl-containing fibers showed reduced mass loss, suggesting that citral inhibits lysozyme action through direct interaction or structural shielding.

- Testing the cytotoxicity of the fibers on the NHDF cell line showed cell viability above the biosafety threshold (>70%), according to ISO 10993-5, confirming their potential for biomedical applications in contact with living tissues.

- Fibers functionalized with 2-FPBA and citral demonstrated antifungal and antibiofilm activity, particularly against *A. brasiliensis*, suggesting a possible synergistic effect of the two aldehydes. The lack of biofilm formation indicates the potential of these materials to act as a physical barrier in preventing wound infection.

Chapter 4. Synthesis and characterization of new eco-friendly food packaging based on imine derivatives of chitosan

- To evaluate the influence of citral-functionalized chitosan on paper properties, commercial white kraft paper samples were coated with two citryl-iminochitosan solutions and a chitosan solution, used as a control. The citryl-iminochitosan solutions used for coating were obtained by the acid condensation reaction of chitosan with citral in different molar ratios of glucosamine/aldehyde units, namely 1/1 and 2/1.

- The formation of citryl-iminochitosan derivatives was confirmed by FTIR spectroscopy through the appearance of the specific band for the imine bond at 1641 cm^{-1}.

- The coated paper had a smoother surface with filled interfibrillar pores, indicating that the coating solution, chitosan or chitosan imine derivative, had penetrated between cellulose fibers on the paper surface. The differences in uniformity observed between samples can be explained by variations in the viscosity of the solutions applied and the possible infiltration of air into the pores.

- Observation of the samples under polarized light revealed that the intense birefringence of the uncoated paper, specific to cellulose fibers, was diminished by the chitosan coating and intensified by the citryl-iminochitosan coating. This observation is consistent with the self-assembly capacity through hydrophobic/hydrophilic segregation of the imine derivative and suggests a physical crosslinking of the chitosan chains anchored on the cellulose fibers through hydrogen bonds and imine units, forming a thin semicrystalline film and contributing to better paper reinforcement.

- The coating of the paper did not significantly affect its thermal stability, with the maximum degradation temperature being shifted very slightly towards higher values due to crosslinking.

- Coating the paper with chitosan or citryl-iminochitosan significantly improved its mechanical properties, increasing tensile strength, elongation, and flexibility by optimizing interfacial adhesion and intermolecular interactions between the cellulose fibers and the coating layer.

- The tensile energy absorption (TEA) index increased substantially, to values twice those of the reference paper, indicating improved resistance to repeated stress, which is important for packaging.

- Coating the paper with citryl-iminochitosan resulted in a significant increase in tear resistance, by approximately 50%, exceeding the performance of simple chitosan, attributed to the reduced viscosity and efficient crosslinking induced by citral.

- Coating with chitosan and citryl-iminochitosan led to significant increases in flexural strength (150-350% compared to 100%), due to the formation of hydrogen bonds and additional intermolecular interactions, favored by the citryl-imine structure.

- Coating with citryl-iminochitosan significantly reduced water adsorption (from 22–26 g/m² to 17–19 g/m²), due to the filling of surface pores and the hydrophobic character conferred by citral.

- The coating significantly increased the hydrophobicity of the surface, with an increase in the water contact angle from 110° to 121°. This is due to the filling of the pores with chitosan and hydrophobic citryl-imine chains, as well as the reorientation of the molecular structure that directs the hydrophobic chains towards the outside.

- The coating significantly reduced the water vapor transmission rate from 400-270 g/m² to 170-150 g/m² due to the hydrophobic nature of the citryl-iminochitosan coatings, which is promising for good food preservation by reducing moisture transfer.

- Experiments on the *in vitro* release of citral have demonstrated the significant influence of the pH of the medium and the solubility of citral: in an acidic medium, release is faster due to the shift of the equilibrium of the reaction towards the reactants, and in ethanol, release is greater than in water due to higher solubility of citral. Thus, release is a dynamic process, maintained by the continuous removal of citral from the release medium.

- The materials obtained were evaluated to determine their ability to extend the shelf life of food, choosing raspberries as the model fruit for the study. It was established that the presence of citral contributed to color stability by reducing browning and degradation processes. The coatings also slowed dehydration and anthocyanin accumulation, delaying ripening and deterioration, and mitigating yellowing/browning.

- Fruit coated with impregnated paper showed greater firmness compared to commercial packaging. The sample with the lowest citral content was the most effective in maintaining fruit firmness.

- Covering the fruit created a barrier against contamination by pathogens by increasing antipathogenic activity by up to approximately two times in the presence of citral.

The original results presented in the thesis were disseminated in the form of ISI scientific articles in national and international journals.

Papers published in ISI-indexed journals

1. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Double functionalization of chitosan based nanofibers towards biomaterials for wound healing*, Reactive and Functional Polymers, 167, 105028 ,**2021,** https://doi.org/10.1016/j.reactfunctpolym.2021.105028 **F.I.$_{2024}$= 5.1**

2. R. Lungu, M. A. Paun, D. Peptanariu, D. Ailincai, L. Marin, M. V. Nichita, V. P. Paun, *Biocompatible Chitosan-Based Hydrogels for Bioabsorbable Wound Dressings*, Gels, 8(2), 107, **2022,** https://doi.org/10.3390/gels8020107 **F.I.$_{2024}$= 5.3**

3. L. Marin, B.-I. Andreica, D. Ailincai, A. Anisiei, **R. Lungu**, *Chitosan: a critical review on structural*

characteristics – properties relationship, Cellulose Chemistry and Technology, 59 (7-8), 747-759, **2025**, **F.I.$_{2024}$= 1.1**.

4. R. Lungu, F. Ciolacu, L. Marin, I. Spiridon, *Chitosan-Citral Coated Paper: A Simple and Sustainable Approach to Eco-Friendly Food Packaging*, Cellulose, (**2025**) **F.I.$_{2024}$= 4.8**, *under review.*

5. R. Lungu, D. Ailincai, M.M. Iftime, I. Rosca, I. Sandu, L. Marin, Hydrogels based on iminodynamers for biomedical applications, **2025**, *manuscript in progress.*

Proceedings

1. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Double-functionalized chitosan nanofibers for wound healing*, Progress in Organic and Macromolecular Compounds Proceedings (MacroIasi2021), October 7-9, **2021**.

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Oral presentations

1. R. Lungu, D. Ailincai, L. Marin, *pH influence on the biodegradation of chitosan based hydrogels*, International Congress of the "Apollonia" University of Iasi „Preparing for the future by promoting excellence", Iasi, February 29 – March 3, **2024**.

2. R. Lungu, D. Ailincai, L. Marin, *An experimental study on chitosan-based hydrogels biodegradation for wound healing*, International Conference Chimia 2024 „New trends in applied chemistry", Constanta, May 30 – June 1, **2024**.

3. R. Lungu, D. Ailincai, L. Marin, *Iminoboronate chitosan hydrogels for bioapplications: synthesis and characterization*, "Chemistry - open frontier to knowledge", 15th edition, Iasi, June 27, **2024**.

4. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Chitosan nanofibers for wound dressings*, International Congress of the "Apollonia" University of Iasi „Preparing for the future by promoting excellence", 35th edition, Iasi, February 27 – March 1, **2025**.

Poster presentation

1. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Double-functionalized chitosan nanofibers for wound healing*, Progress in Organic and Macromolecular Compounds (MacroIasi 2021), October 7-9, **2021**.

2. R. Lungu, L. Marin, I. Spiridon, F. Ciolacu, *Chitosan citryl-imine derivatives used as coatings for food packaging*, International Congress of the "Apollonia" University of Iasi „Preparing for the future by promoting excellence", Iasi, February 29 – March 3, **2024**.

3. R. Lungu, L. Marin, I. Spiridon, F. Ciolacu, *Coating formulations based on imine chitosan derivatives used in food packaging*, International Conference Chimia 2024 „New trends in applied chemistry", Constanta, May 30 – June 1, **2024**.

4. R. Lungu, L. Marin, I. Spiridon, F. Ciolacu, *New eco-friendly food packaging based on imine-chitosan derivatives*, "Chemistry - an open frontier to knowledge", 15th edition, Iasi, June 27, **2024**.

5. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Design of biocompatible chitosan nanofibers for wound healing*, IasiCHEM 2024 Conference, 6th edition, Iasi, October 31 - November 1, **2024.**

6. R. Lungu, A. Anisiei, I. Rosca, A.I. Sandu, D. Ailincai, L. Marin, *Modified chitosan nanofifers for bioabsorbable wounds dressing*, Open door to the future scientific communications of young researchers (MacroYouth2024), 5th edition, November 15, **2024.**

Selective bibliography

[1] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (2008) 314–321. https://doi.org/10.1038/nature07039.

[2] A. Shpichka, D. Butnaru, E.A. Bezrukov, R.B. Sukhanov, A. Atala, V. Burdukovskii, Y. Zhang, P. Timashev, Skin tissue regeneration for burn injury, Stem Cell Res Ther 10 (2019) 94. https://doi.org/10.1186/s13287-019-1203-3.

[3] A.H. Hall, L. Mathieu, H.I. Maibach, Acute chemical skin injuries in the United States: a review, Crit Rev Toxicol 48 (2018) 540–554. https://doi.org/10.1080/10408444.2018.1493085.

[4] O. Yazarlu, M. Iranshahi, H.R.K. Kashani, S. Reshadat, S. Habtemariam, M. Iranshahy, M. Hasanpour, Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review, Pharmacol Res 174 (2021) 105841. https://doi.org/10.1016/j.phrs.2021.105841.

[5] P. Ghandforoushan, N. Golafshan, F. Babu Kadumudi, M. Castilho, A. Dolatshahi-Pirouz, G. Orive, Injectable and adhesive hydrogels for dealing with wounds, Expert Opin Biol Ther 22 (2022) 519–533. https://doi.org/10.1080/14712598.2022.2008353.

[6] A. Anisiei, F. Oancea, L. Marin, Electrospinning of chitosan-based nanofibers: from design to prospective applications, Reviews in Chemical Engineering 39 (2023) 31–70. https://doi.org/10.1515/revce-2021-0003.

[7] M.S. Kamińska, A.M. Cybulska, K. Skonieczna-Żydecka, K. Augustyniuk, E. Grochans, B. Karakiewicz, Effectiveness of Hydrocolloid Dressings for Treating Pressure Ulcers in Adult Patients: A Systematic Review and Meta-Analysis, Int J Environ Res Public Health 17 (2020) 7881. https://doi.org/10.3390/ijerph17217881.

[8] H. Liu, C. Wang, C. Li, Y. Qin, Z. Wang, F. Yang, Z. Li, J. Wang, A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing, RSC Adv 8 (2018) 7533–7549. https://doi.org/10.1039/C7RA13510F.

[9] S. Alven, B.A. Aderibigbe, Chitosan and Cellulose-Based Hydrogels for Wound Management, Int J Mol Sci 21 (2020) 9656. https://doi.org/10.3390/ijms21249656.

[10] R. Golmohammadi, S. Najar-Peerayeh, T. Tohidi Moghadam, S.M.J. Hosseini, Synergistic Antibacterial Activity and Wound Healing Properties of Selenium-Chitosan-Mupirocin Nanohybrid System: An in Vivo Study on Rat Diabetic Staphylococcus aureus Wound Infection Model, Sci Rep 10 (2020) 2854. https://doi.org/10.1038/s41598-020-59510-5.

[11] S. Ono, R. Imai, Y. Ida, D. Shibata, T. Komiya, H. Matsumura, Increased wound pH as an indicator of local wound infection in second degree burns, Burns 41 (2015) 820–824. https://doi.org/10.1016/j.burns.2014.10.023.

[12] A. Anisiei, I. Rosca, A.-I. Sandu, A. Bele, X. Cheng, L. Marin, Imination of Microporous Chitosan Fibers - A Route to Biomaterials with “On Demand” Antimicrobial Activity and Biodegradation for Wound Dressings, Pharmaceutics 14 (2022) 117. https://doi.org/10.3390/pharmaceutics14010117.

[13] L. Marin, M. Popa, A. Anisiei, S.-A. Irimiciuc, M. Agop, T.-C. Petrescu, D. Vasincu, L. Himiniuc, A Theoretical Model for Release Dynamics of an Antifungal Agent

Covalently Bonded to the Chitosan, Molecules 26 (2021) 2089. https://doi.org/10.3390/molecules26072089.

[14] M.-M. Iftime, S. Morariu, L. Marin, Salicyl-imine-chitosan hydrogels: Supramolecular architecturing as a crosslinking method toward multifunctional hydrogels, Carbohydr Polym 165 (2017) 39–50. https://doi.org/10.1016/j.carbpol.2017.02.027.

[15] B.-I. Andreica, D. Ailincai, A.-I. Sandu, L. Marin, Amphiphilic chitosan-g-poly(trimethylene carbonate) – A new approach for biomaterials design, Int J Biol Macromol 193 (2021) 414–424. https://doi.org/10.1016/j.ijbiomac.2021.10.174.

[16] L. Marin, D. Ailincai, M. Mares, E. Paslaru, M. Cristea, V. Nica, B.C. Simionescu, Imino-chitosan biopolymeric films. Obtaining, self-assembling, surface and antimicrobial properties, Carbohydr Polym 117 (2015) 762–770. https://doi.org/10.1016/j.carbpol.2014.10.050.

[17] F. Hong, P. Qiu, Y. Wang, P. Ren, J. Liu, J. Zhao, D. Gou, Chitosan-based hydrogels: From preparation to applications, a review, Food Chem X 21 (2024) 101095. https://doi.org/10.1016/j.fochx.2023.101095.

[18] D. Ailincai, L. Mititelu-Tartau, L. Marin, Citryl-imine-PEG-ylated chitosan hydrogels – Promising materials for drug delivery applications, Int J Biol Macromol 162 (2020) 1323–1337. https://doi.org/10.1016/j.ijbiomac.2020.06.218.

[19] K. Sharma, S. Guleria, V.K. Razdan, V. Babu, Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds, Ind Crops Prod 154 (2020) 112569. https://doi.org/10.1016/j.indcrop.2020.112569.

[20] D. Ailincai, L. Marin, S. Morariu, M. Mares, A.-C. Bostanaru, M. Pinteala, B.C. Simionescu, M. Barboiu, Dual crosslinked iminoboronate-chitosan hydrogels with strong antifungal activity against Candida planktonic yeasts and biofilms, Carbohydr Polym 152 (2016) 306–316. https://doi.org/10.1016/j.carbpol.2016.07.007.

[21] L. Martinengo, M. Olsson, R. Bajpai, M. Soljak, Z. Upton, A. Schmidtchen, J. Car, K. Järbrink, Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies, Ann Epidemiol 29 (2019) 8–15. https://doi.org/10.1016/j.annepidem.2018.10.005.

[22] A.S. Ribeiro, S.M. Costa, D.P. Ferreira, R.C. Calhelha, L. Barros, D. Stojković, M. Soković, I.C.F.R. Ferreira, R. Fangueiro, Chitosan/nanocellulose electrospun fibers with enhanced antibacterial and antifungal activity for wound dressing applications, React Funct Polym 159 (2021) 104808. https://doi.org/10.1016/j.reactfunctpolym.2020.104808.

[23] A. Anisiei, B.-I. Andreica, L. Mititelu-Tartau, C.G. Coman, R. Bilyy, G. Bila, I. Rosca, A.-I. Sandu, E. Amler, L. Marin, Biodegradable trimethyl chitosan nanofiber mats by electrospinning as bioabsorbable dressings for wound closure and healing, Int J Biol Macromol 249 (2023) 126056. https://doi.org/10.1016/j.ijbiomac.2023.126056.

[24] The Commission Of The European Communities, Commission regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, Https://Eur-Lex.Europa.Eu/LexUriServ/LexUriServ.Do?Uri=OJ:L:2009:135:0003:0011:EN:PDF (2009).

[25] A. Boonmahitthisud, C. Booranapunpong, C. Pattaradechakul, S. Tanpichai, Development of water-resistant paper using chitosan and plant-based wax extracted from banana leaves, Int J Biol Macromol 240 (2023) 124412. https://doi.org/10.1016/j.ijbiomac.2023.124412.

[26] I. Yoplac, L. Vargas, P. Robert, A. Hidalgo, Characterization and antimicrobial activity of microencapsulated citral with dextrin by spray drying, Heliyon 7 (2021) e06737. https://doi.org/10.1016/j.heliyon.2021.e06737.