RSC Pharmaceutics

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Pharm.*, 2025, **2**, 1500

Biopolymeric films based on chitosan and copaiba oleoresin: preliminary insights for oral applications

Taleessa Vieira Gomes,[a] Ana Barbara Polo,[b] Mariana Simões de Oliveira,[a] Beatriz Vilela Tomé,[d] Daphne Fonseca de Coppoli Lanferini,[e] Laura Lima de Queiroz,[f] Sabrina França Silva,[f] Antonio Carlos Sant'ana,[e] Diogo Montes Vidal,[f] Guilherme Diniz Tavares[b,c] and Ana Carolina Morais Apolônio *[a,g]

Polymeric films for oral applications remain underexplored, despite their potential as multifunctional therapeutic systems. Effective oral dressings must adhere to mucosal surfaces, promote tissue repair, reduce microbial proliferation, and protect injured areas. This study focused on the formulation and characterization of bioactive films composed of chitosan (CH) and copaiba oleoresin (COR) at varying concentrations (0.5%, 1%, and 3.5%), selected based on prior research. The films were obtained by solvent evaporation and evaluated for their physicochemical, morphological, and biological properties. Spectroscopic analyses (Raman and FT-IR) revealed molecular interactions between CH, COR, and excipients, with significant spectral shifts in functional groups, particularly from malic acid and glycerin, indicating successful incorporation.The films exhibited a moisture content ranging from 15.24% to 20.23%, which decreased with higher COR concentrations due to their hydrophobic nature. While all formulations demonstrated high swelling capacity and solubility, increased COR content reduced water absorption, suggesting a concentration-dependent modulation of film hydrophobicity. Given these findings, no single formulation emerged as universally optimal; instead, the selection of COR concentration should align with specific therapeutic goals, such as rapid bioactive release or prolonged mucosal adhesion. These results highlight the potential of CH–COR films as promising candidates for the topical treatment of oral mucosal lesions. Future research should focus on optimizing film composition to enhance antimicrobial efficacy while maintaining mechanical stability, paving the way for clinical applications.

Received 30th April 2025,
Accepted 22nd July 2025

DOI: 10.1039/d5pm00127g

rsc.li/RSCPharma

1. Introduction

Oral ulcerative conditions, particularly oral mucositis (OM), represent a significant clinical challenge, especially among patients undergoing chemoradiotherapy. OM is characterized by painful ulcerations, impaired oral function, secondary infections, and increased healthcare costs, ultimately compromising patient adherence to treatment and quality of life.[1]

Despite its clinical impact, current therapeutic options for OM remain limited. The majority of available strategies focus either on prophylaxis or symptomatic relief, such as pain control.[1,2] However, there is a lack of effective bioactive treatments capable of simultaneously promoting mucosal repair, mitigating microbial proliferation, and modulating inflammation, underscoring the need for innovative, multifunctional therapeutic platforms.

Recent advances in biomaterials science have accelerated the development of bioactive polymeric films as platforms for site-specific drug delivery. Such systems offer significant advantages, including bioadhesion, biocompatibility, controlled release, and mechanical flexibility, which are particularly relevant for intraoral applications.[3,4] These features may also contribute to improved functionality, safety, and patient compliance, since mucoadhesive films can adhere to the lesion sites, avoid displacement by saliva or food, and reduce the need for frequent application. By acting as protective

[a]*Postgraduate Program in Dentistry, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil. E-mail: carolina.apolonio@ufjf.br*
[b]*Postgraduate Program in Pharmaceutical Sciences, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*
[c]*Institute of Pharmacology, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*
[d]*School of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*
[e]*Postgraduate Program in Chemistry, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*
[f]*Department of Chemistry, ICEx, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*
[g]*Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*

dressings that also deliver active compounds, these systems hold the potential to enhance therapeutic outcomes in a comfortable, localized, and non-invasive manner.

Among available biopolymers, chitosan (CH)—a cationic polysaccharide derived from chitin deacetylation—has garnered attention due to its intrinsic antimicrobial activity, mucoadhesiveness, and film-forming capability.[3–6] These features make CH highly suitable for the design of mucoadhesive delivery systems targeting dynamic and moist environments such as the oral cavity.

Copaiba oleoresin (COR), a natural secretion obtained from *Copaifera* species, has been widely recognized for its pharmacological potential, particularly in anti-inflammatory, antimicrobial, antioxidant, and wound-healing applications.[7–10] Chemically, COR is mainly composed of terpenes and sesquiterpenes, with β-caryophyllene being its major bioactive component.[9] Studies have shown that β-caryophyllene exerts anti-inflammatory effects *via* cannabinoid receptor activation (CB2), modulating immune responses and reducing inflammatory mediators.[8] Additionally, the antimicrobial activity of COR has been reported against pathogenic microorganisms commonly associated with oral infections.[10] Its hydrophobic nature also suggests potential benefits in film formulations, improving moisture resistance and controlled release of bioactive compounds.[7–10] However, the incorporation of COR into CH-based films has not yet been explored for oral therapeutic purposes, representing a critical knowledge gap.

In this context, the present study proposes an exploratory formulation and preliminary physicochemical characterization of CH-based polymeric films containing COR. To our knowledge, this is the first study to investigate the feasibility of integrating these two biocompatible agents into a single matrix intended for oral biomedical applications. The use of biopolymers and natural compounds in an integrated system offers a promising and sustainable alternative for the development of new pharmaceutical treatments, which can be applied in various health care areas, from infection treatment to wound healing.[12–19]

This pilot study evaluates the morphological, structural, and antimicrobial characteristics of the resulting films, providing foundational data for future optimization and biological validation. By aligning with the current trend of developing multifunctional, plant-based polymeric systems, this work contributes to the expanding body of literature focused on eco-friendly, bioactive therapeutic platforms. The findings presented here aim to support future translational research and guide the rational design of advanced oral delivery systems for the management of ulcerative lesions and mucosal healing.

2. Materials and methods

2.1 *Copaifera officinalis* oleoresin chemical composition

The COR (*Copaifera officinalis* oleoresin) used in the analyses was acquired from Terra Flor® (Vila Velha, ES, Brazil) and is registered with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under no. A2B7469. For the analysis of COR, 10 μL aliquots were diluted in ethyl acetate and subjected to gas chromatography–mass spectrometry (GC-MS) (GC-2010, Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer (QP2010 Plus, Shimadzu Corporation, Kyoto, Japan). The analysis was performed on a non-polar capillary column (Equity-5, Supelco®, 5% diphenyl/95% dimethylsiloxane). The oven's initial temperature was set at 50 °C for 3 minutes and then increased at 7 °C min^{-1} to 250 °C, where it was held for 10 minutes. The injector and interface temperatures were maintained at 250 °C, and the ion source temperature at 200 °C. Mass spectra were interpreted using standard data libraries from the NIST Mass Spectrometry Data Center, Adams[20] and Flavors and Fragrances of Natural and Synthetic Compounds, with retention indices calculated by the Van den Dool and Kratz method using a standard solution of *n*-alkanes for reference values.[21]

2.2 Development of CH-based films with COR incorporation

The chitosan used in this study had a low molecular weight and a degree of deacetylation between 75% and 85% (Sigma-Aldrich, St Louis, MO, USA). The mucoadhesive film formulation based on CH and incorporated with COR was developed using the solvent evaporation technique, following modified methodologies from Aksungur *et al.*[19] and Cazón *et al.*[22] The precursor solution was prepared by dissolving 2% (w/v) CH in a 2% (v/v) malic acid solution (Synth, Diadema, SP, Brazil). This mixture was stirred mechanically at 1200 rpm (Fisatom® 711DS, São Paulo, Brazil) for 1 hour at room temperature (~25 °C). Then, 10% (v/v) bidistilled glycerin (Vetec, Rio de Janeiro, RJ, Brazil) was added as a plasticizer, and the solution was stirred for an additional 30 minutes. During this stage, 2% (v/v) Tween 80 (Sigma-Aldrich, St Louis, MO, USA) was incorporated to improve the dispersion of the hydrophobic COR within the aqueous polymer matrix.

After 1 hour and 30 minutes of stirring, the resulting 78 mL of film-forming solution was divided equally into four beakers. Different concentrations of COR (0.5%, 1%, and 3.5%) were manually added to three of them, and one served as a control without COR. Each formulation (18.6 mL) was poured into glass Petri dishes (90 × 15 mm) and subjected to an ultrasonic bath (Ultronic – Unique, USC – 2800, Indaiatuba, SP, Brazil) for 1 hour to remove air bubbles. The films were dried in an oven (Nova Instruments – NI 1512, Piracicaba, SP, Brazil) at 50 °C for 24 hours.

2.3 Determination of zeta potential (PZ) and hydrodynamic diameter (DHm) of precursor solutions for CH–COR films

The Zeta Potential (PZ) and Hydrodynamic Diameter (DHm) were assessed using dynamic light scattering (DLS) at a laser incidence angle of 173° relative to the sample. PZ was determined *via* electrophoretic mobility analysis. Each precursor solution was diluted 40-fold using purified water. Measurements were performed at room temperature (25 ± 1 °C) using a Zetasizer Nanoseries (Malvern Instruments,

Worcestershire, UK), with the results presented as the average of three independent measurements.

2.4 Physicochemical characterization of CH–COR films

2.4.1 Determination of average weight. The average weight of the films was determined by weighing four 1.0 cm × 1.0 cm samples from films (n = 3) prepared at different times for each concentration using an analytical balance. Results were recorded as mean ± standard deviation.[23]

2.4.2 Determination of thickness. The thickness of films was measured with a digital caliper on samples (n = 3) from three films, each prepared at different times, for each concentration. Values were recorded and expressed as the mean ± standard deviation.[24]

2.4.3 Determination of moisture content. Moisture content was determined using four 1.0 cm × 1.0 cm samples from films (n = 3) that were weighed (W_{wet}) and dried in an oven at 60 °C ± 2 °C for 24 hours. The films were weighed (W_{dry}) again, and moisture content was calculated using eqn (1), where W_{wet} is the mass of the film and W_{dry} is the mass of the dried film.[23] Results were expressed as mean ± standard deviation.

$$\text{Moisture } (\%) = (W_{wet} - W_{dry}) \times 100/W_{wet} \quad (1)$$

2.4.4 Determination of swelling degree. The swelling degree was determined by immersing three 1.0 cm × 1.0 cm samples of films (n = 3) from each formulation in artificial saliva[25] (pH 6) at 37 °C for 24 hours. The samples were weighed at 1 h, 4 h, 6 h, 8 h, 10 h, and 24 h after excess water removal with filter paper. The swelling degree was calculated using formula (2), where $P0$ is the initial weight and PI is the swollen weight.[26] Results were expressed as mean ± standard deviation.

$$\text{Swelling } (\%) = (\text{PI} - P0) \times 100/P0 \quad (2)$$

2.4.5 Determination of solubility. Solubility was assessed using three samples from each film (n = 3), and the concentration was tested for swelling. After the swelling test, the films were dried in an oven at 60 °C for 24 hours and weighed. The solubility percentage was calculated using formula (3), where $P0$ is the initial weight and PD is the post-drying weight.[23] Results were expressed as mean ± standard deviation.

$$\text{Solubility } (\%) = (P0 - \text{PD}) \times 100/P0 \quad (3)$$

2.5 Vibrational electronic Raman spectroscopy and Fourier transform infrared spectroscopy (FT-IR)

To analyze the molecular interactions present in the formulated films relative to their pure constituents, Raman spectroscopy was utilized. Samples of the films and their isolated constituents (chitosan, malic acid, glycerin, and Tween 80) were analyzed using an FT-Raman Bruker-RFS-100 spectrometer equipped with a liquid nitrogen-cooled germanium detector, coupled to an Olympus microscope with a 40× long-range magnification lens. The laser excitation line was Nd-YAG with a wavelength of 1064 nm and a power of 400 mW, with 512 scans. The films were also analyzed by FT-IR to complement the Raman spectroscopy results using a Bruker Vertex 70 spectrophotometer with an Attenuated Total Reflectance (ATR) mode and a diamond crystal. Spectra were obtained as an average of 512 consecutive scans with a resolution of 4 cm^{-1}. Data from the spectra generated by both methodologies were exported and analyzed using OriginPro 2018 software.

2.6 Scanning electron microscopy (SEM)

The evaluation of the surface morphology was performed using scanning electron microscopy (SEM) under low vacuum conditions. Images were obtained using a FEI QUANTA 250 scanning electron microscope at the microscopy laboratory of the Federal University of Juiz de Fora. Magnifications of 200× and 500× were applied.

2.7 Biological evaluation of CH–COR films

2.7.1 Cell viability assay. The cytotoxicity assay was conducted following Genesi *et al.* (2023),[18] with modifications, using the MTT reduction assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). J774A.1 cells (a commonly used murine macrophage cell line derived from reticulum cell sarcoma) were cultured in RPMI 1640 medium supplemented with fetal bovine serum and an antibacterial solution containing ampicillin and streptomycin. For each well, 2 × 10^5 cells were added and incubated for 20 hours at 37 °C. The assay was performed in 96-well plates, with triplicate for each condition. The tested samples included films with a diameter of 5 mm at concentrations of 0.5%, 1%, and 3.5%, as well as a control (without COR). Additionally, concentrations of COR (75 to 4.69 μg ml^{-1}), CH (1.2 to 0.15 mg ml^{-1}), and the combination of COR + CH at the same concentrations were tested. These concentrations were defined based on previous minimum inhibitory concentration (MIC) assays[11] and include the concentration range of the active ingredients present in each film disc. All film discs were previously sterilized by UV light exposure for 15 minutes on each side. The plates were then incubated for 48 hours at 37 °C, followed by the addition of MTT at a concentration of 5 mg mL^{-1}. After further incubation, absorbance was measured using a spectrophotometer at 570 nm.

2.7.2 Antimicrobial activity. The assay was performed to measure the sensitivity of oral microorganisms to CH–COR films using Petri dishes containing Mueller–Hinton agar, with a standardized height of 5 mm, to evaluate the activity against the following microbial strains: *Streptococcus sobrinus* (ATCC 27351); *Streptococcus sanguinis* (ATCC 10556); *Enterococcus faecalis* (ATCC 19433, ATCC 51299); *Enterococcus faecium* (ATCC 6569); *Aggregatibacter actinomycetemcomitans* (ATCC 29522); *Streptococcus mutans* (ATCC 25175); *Streptococcus oralis* (ATCC 10557); *C. albicans* (clinical isolate); *Candida tropicalis* (ATCC 750); *Staphylococcus aureus* (ATCC 33591, ATCC 25904, ATCC BAA977); *Streptococcus pyogenes* (ATCC 19615); *Acinetobacter baumannii* (ATCC 19606); and *Klebsiella pneumoniae* (ATCC BAA2814). Microbial inocula were prepared using a McFarland

turbidity scale of 0.5, equivalent to a concentration of 10^8 CFU per mL for bacterial inocula and 10^6 CFU per mL for fungal inocula. Films at concentrations of 0.5%, 1%, 3.5%, and the control (without COR), with diameters of 5 mm, were previously sterilized under UV light for 15 minutes on each side and placed on the inoculated plates. In addition to the films, COR at the respective concentrations as those incorporated into the films, 2% of CH solution, and 2% of malic acid solution were tested. For the positive control, 2% of chlorhexidine was used. For all these active ingredients, 10 μL of aliquots were used on absorbent paper discs with the same diameter as the films. All tested actives/films were positioned on the plates according to a predefined map. The test was performed in triplicate. The plates were incubated for 24 hours at 37 °C under microaerophilic or aerobic atmospheres, depending on the requirements of each microorganism. After this period, the inhibition halos formed around each sample were measured using a ruler specifically used for the Kirby–Bauer test.[27]

2.7.3 Statistical analysis. Statistical analysis was performed using Jamovi software (version 2.2, The Jamovi Project, Sydney, Australia). Non-parametric tests were employed to evaluate differences between experimental groups and repeated measures across time points. For the swelling analysis, intergroup comparisons were performed using the Kruskal–Wallis test to assess whether the concentration of copaiba oleoresin influenced swelling behavior. Intragroup comparisons across different time points (1 h to 24 h) were conducted using the Friedman test to determine whether swelling varied significantly over time. Results were expressed as means and standard deviations, and statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1 Determination of terpenic compounds

The GC-MS analysis' results revealed the presence of various sesquiterpenes in the sample, as shown in Table 1, highlighting *trans*-caryophyllene, which accounted for 35.5% of the total composition. This compound was identified as the main component of the analyzed COR, aligning with the literature where *trans*-caryophyllene is often recognized as a chemical marker in *Copaifera* species across the collected 40 COR samples.[28] The high concentration of *trans*-caryophyllene is particularly noteworthy due to its known anti-inflammatory, antioxidant, and analgesic properties,[29,30] which could positively influence the therapeutic properties of the film, such as its ability to modulate inflammatory responses and to protect against oxidative stress. The precise identification of these compounds is essential to ensure that the final formulation delivers the desired therapeutic effects, as variations in chemical composition can directly impact the performance of the developed products. Findings like those of Gelmini *et al.* (2013)[31] suggest that the chemical composition of COR can significantly vary depending on the species and environmental conditions, as seen in *Copaifera langsdorffii Desf*, in which β-bergamotene was the main constituent. Differences between species may explain the significant variation in chemical composition, as their chemical constituents can vary according to the species within the genus, as well as seasonal and climatic characteristics, soil type and composition, and rainfall index.[30]

3.2 Development of CH-based films with COR incorporated

Given COR's chemical composition, the films' formulation and characterization were developed. CH (2%) films formulated with different concentrations of COR exhibited variations in color, uniformity, and opacity, both before and after the drying process (Fig. 1). Tween 80 was selected for its surfactant and emulsifying properties. The addition of Tween 80 was necessary to facilitate the dispersion of copaiba oleoresin (COR) within the chitosan matrix due to its hydrophobic nature. The use of this emulsifier is supported by previous studies demonstrating its effectiveness in enhancing the homogeneity, stability, and functionality of bioactive polymeric films.[32–34]

Chu *et al.* (2019)[32] employed Tween 80 to incorporate cinnamon essential oil into pullulan films, maintaining their antioxidant and antimicrobial properties. Darbasi *et al.* (2017)[33] reported improved dispersion of antioxidants in chitosan-based films, while Biliuta *et al.* (2025)[34] demonstrated the safety and absence of cytotoxicity when using Tween 80 in polymeric films containing terbinafine. Therefore, the inclusion of Tween 80 in this formulation is justified by its technological effectiveness and compatibility with topical oral applications.

Initially, the films displayed a clear and uniform color with slight translucency, especially at the 0.5% concentration. Higher concentrations (1% and 3.5%) maintained a homogeneous appearance, with slightly greater opacity. After drying, the 0.5% films remained translucent and homogeneous, while the 1% and 3.5% films became opaque and had a more intense color. These results indicate that the concentration of COR directly influences the visual properties of CH films, with higher opacity and color intensity. Transparency is an important characteristic, especially for wound dressings, including mucositis, because it allows continuous visual assessment of the wound without needing to remove the dressing. This facilitates monitoring the progression of healing.[35] Paranhos *et al.* (2022)[36] produced films with CH and copaiba oil, which also showed an increase in color intensity with higher concentrations of copaiba oil, becoming stronger and more yellow. This is different from the present work, which became opaque and whiter. The study did not specify the species used but described the oil as yellow, greenish, and brownish, different from the oleoresin used in our work, which is light, slightly yellow, and translucent, which may explain the difference in hue.

3.3. Characterization of the precursor solutions of CH–COR films

3.3.1 Determination of zeta potential (ZP) and hydrodynamic diameter medium (DHm) of precursor solutions. The

Table 1 Identification of compounds and the concentration range of COR constituents by GC/MS

No.	Compounds	Class	% area	RI exp.	RI lit.
1	α-Cubebene	Sesquiterpene	1.2	1461	1461
2	δ-Elemene	Sesquiterpene	0.7	1475	1472
3	α-Copaene	Sesquiterpene	6.9	1497	1497
4	β-Cubebene	Sesquiterpene	0.8	1544	1544
5	α-Bergamotene	Sesquiterpene	7.3	1591	1592
6	β-Elemene	Sesquiterpene	1.7	1597	1596
7	*trans*-Caryophyllene	Sesquiterpene	35.5	1606	1606
8	unk. 1 (105/91/107/93/161)	Sesquiterpene	0.5	1652	
9	β-*trans*-Farnesene	Sesquiterpene	0.6	1672	1672
10	α-Humulene	Sesquiterpene	7.0	1679	1679
11	γ-Muurolene	Sesquiterpene	0.8	1696	1695
12	Germacrene D	Sesquiterpene	2.4	1719	1719
13	γ-Muurolene	Sesquiterpene	7.7	1724	1725
14	β-Selinene	Sesquiterpene	1.1	1729	1729
15	β-Bisabolene	Sesquiterpene	5.6	1736	1736
16	Bicyclogermacrene	Sesquiterpene	0.6	1744	1744
17	δ-Cadinene	Sesquiterpene	3.5	1765	1765
18	γ-Cadinene	Sesquiterpene	0.6	1767	1767
19	β-Sesquiphellandrene	Sesquiterpene	0.7	1777	1777
20	(*E*)-α-Bisabolene	Sesquiterpene	0.6	1779	1783
21	Germacrene B	Sesquiterpene	2.0	1840	1844
22	Caryophyllene oxide	Sesquiterpene	1.0	2001	2001
23	Caryophyllenol	Sesquiterpene	0.5	2069	2073
24	unk. 2 (109/81/161/95/204)	Sesquiterpene	1.0	2077	
25	τ-Muurolol	Sesquiterpene	0.6	2202	2205
26	δ-Cadinol	Sesquiterpene	0.7	2214	2219
27	α-Cadinol	Sesquiterpene	0.8	2250	2251
28	unk. 3 (95/43/107/121/93)	Diterpene	0.5	2594	
29	unk. 4 (95/107/189/121/81)	Diterpene	1.7	2739	
		Total	93.9%		

unk.: unknown chemical structure.

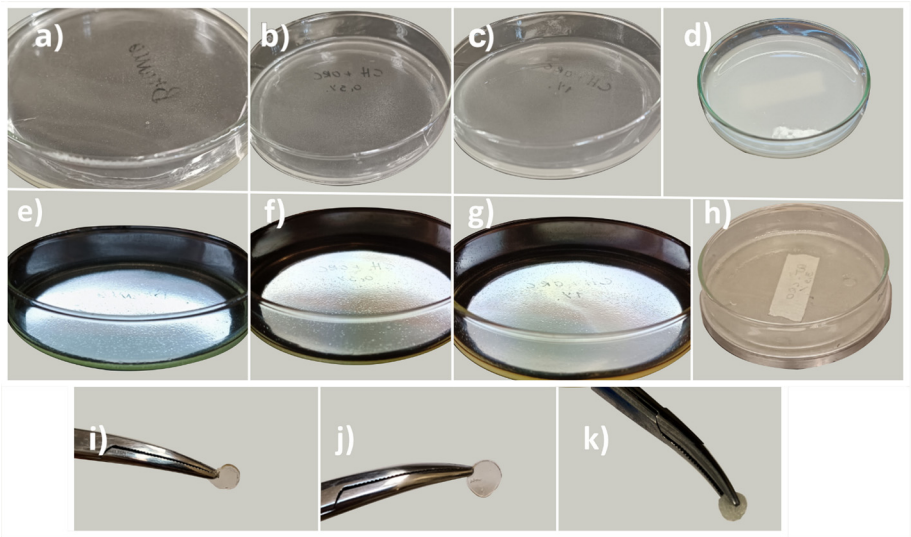

Fig. 1 Films based on CH (2%) and COR at different concentrations (0.5%, 1%, and 3.5%) and the blank before and after drying. (a) Control pre-drying; (b) 0.5% pre-drying; (c) 1% pre-drying; (d) 3.5% pre-drying; (e) control post-drying; (f) 0.5% post-drying; (g) 1% post-drying; (h) 3.5% post-drying; (i) 0.5% film cut; (j) 1% film cut; and (k) 3.5% film cut.

zeta potential (ZP) is a crucial metric for assessing the stability of suspensions, emulsions, and colloidal solutions, as it reflects the degree of electrostatic repulsion between particles, indicating their tendency to aggregate or disperse. Values around ±30 mV are considered indicative of a stable suspension, as such charges prevent particle aggregation.[37,38]

The analyses performed on the precursor solutions of the films, presented in Table 2, show significant variation in both

Table 2 ZP and DHm of the precursor solutions of the films with different concentrations of COR

	0% control film	0.5% film	1% film	3.5% film	*p*-Value
Hydrodynamic diameter (nm)	676.30 ± 154.28	153.43 ± 19.13	573.53 ± 156.30	346.33 ± 13.08	0.024
Zeta potential (mV)	47.07 ± 0.42	11.47 ± 1.12	12.70 ± 2.40	11.63 ± 1.15	0.084

ZP and DHm depending on the different concentrations of COR. The control solution exhibited the highest ZP value; however, none of the evaluated samples reached values close to ±30 mV, the range associated with optimal colloidal stability. Various factors can affect ZP, such as pH and ionic strength. High concentrations of ions, for example, can reduce ZP, leading to solution instability.[39]

This study had an exploratory nature and aimed to establish the feasibility of formulating chitosan- and copaiba oleoresin-based polymeric films. Thus, the analysis focused on the physicochemical and functional characterization of the films, without the application of inferential statistical tests.

The analyses presented in Table 2 show that the control solution (without COR) exhibited the highest values of ZP and DHm when compared to the formulations containing COR (0.5%, 1%, and 3.5%). Although the differences in ZP among groups approached no statistical significance (Kruskal–Wallis, $p = 0.084$), only the DHm values showed a statistically significant difference between the formulations ($p = 0.024$). This behavior may be attributed to the absence of hydrophobic compounds in the control, which allows greater exposure of protonated amino groups ($-NH_3^+$) from chitosan, leading to a higher positive surface charge.[6] The presence of Tween 80 in the control formulation does act as a surfactant and contributes to dispersion; however, without the interference of a lipophilic phase such as COR, the polymer–surfactant system remains more hydrated and electrostatically charged, resulting in higher ZP.

Furthermore, the higher DHm in the control could be related to greater hydration and swelling of chitosan chains in the aqueous medium, favoring the formation of larger polymeric aggregates, especially in the absence of hydrophobic interference. In contrast, when COR is added, it can interact with the chitosan matrix through hydrophobic and van der Waals interactions, leading to partial shielding or neutralization of surface charges and a more compact and stabilized colloidal structure, reducing both ZP and DHm.

However, it is crucial to remember that ZP and DHm are particularly relevant parameters for colloidal systems but may not directly reflect the stability or final properties of solid films. In polymeric films, the interactions between formulation components and the polymer matrix's structure play a more decisive role in the stability and final performance of the product. Thus, the apparent destabilization of the precursor solutions does not necessarily imply a negative impact on the quality and performance of the solid films formed.

Results from Suzuki *et al.* (2007)[40] indicate significant differences in surface charge between water-soluble chitosan (WSC) films and amylose films containing WSC. WSC films showed a very small, positive zeta potential, indicating that while the surface is slightly positively charged, the exposure of amino groups is limited. In contrast, amylose films incorporating WSC showed a higher positive zeta potential, suggesting greater exposure of amino groups on the surface. Thus, combining amylose with CH was able to enhance the surface charge of the films.

In summary, while the ZP and DHm analyses provide valuable insights into the behavior and stability of the precursor solutions, these parameters may not directly correlate with the final film properties. The interactions between chitosan, COR, and other components within the solid film matrix play a more crucial role in determining the overall stability and performance.

3.4. Physicochemical characterization of CH–COR films

3.4.1 Determination of polymeric film's weight and thickness. The average weight and thickness are critical parameters for the quality of the films, as they can influence the material's flexibility, adherence and handling. Consistency in weight and thickness indicates uniformity in film production, ensuring predictable behavior and performance during application.[41] This uniformity minimizes variability, allowing the film to meet the necessary standards for its intended use, making it suitable for biomedical applications, such as the treatment of oral ulcers. The results presented in Table 3 show distinct patterns in weight and thickness across the different COR concentrations. The film containing 3.5% copaiba oleoresin (COR) exhibited the highest weight (0.041 g), while the film with 0.5% COR showed the greatest thickness (0.33 mm). Interestingly, the control film (0%) demonstrated intermediate values for both parameters, with 0.035 g in weight and 0.30 mm in thickness. Although differences in weight and thickness were observed among the formulations, the Kruskal–Wallis test revealed no statistically significant differences between groups for thickness ($p = 0.571$) or weight ($p = 0.128$). These variations reflect the complex interactions between COR and the chitosan matrix, which influence both

Table 3 Weight and thickness of the CH + COR films with means and standard deviations (SD)

	Weight ± SD (g)	Thickness ± SD (mm)
0% control film	0.035 ± 0.00	0.30 ± 0.10
0.5% film	0.025 ± 0.01	0.33 ± 0.06
1% film	0.030 ± 0.00	0.27 ± 0.06
3.5% film	0.041 ± 0.02	0.27 ± 0.06
p-Value	0.128	0.571

the structural organization and the solid content of the resulting films.

At low concentrations of COR (0.5% and 1%), the oleoresin likely disperses more uniformly within the polymeric network, potentially filling voids or reorganizing the matrix in a way that promotes compactness. This could explain the reduced weight at 0.5% (0.025 g) and 1% (0.030 g), despite the increased thickness at 0.5%. A similar phenomenon was described by Sutharsan *et al.* (2022)[42] in chitosan films containing epoxy-activated agarose (EAA), where low additive levels filled interstitial gaps without significantly altering the mass or creating dense agglomerates. Thus, the swelling of the matrix observed at 0.5% COR might not correspond to an actual increase in compact material but rather to network rearrangement that traps more water during film casting and drying.

In contrast, the control film (0%) contained no hydrophobic phase, relying entirely on chitosan and Tween 80. This likely led to the formation of a more cohesive and hygroscopic matrix, as reported by Liu *et al.* (2020)[43] in pure chitosan films, where higher solid content and water retention increased thickness. The intermediate weight of the control film (0.035 g) is consistent with this denser matrix that retains moisture but lacks the mass contribution from oleoresin.

At the highest concentration (3.5% COR), the increased weight (0.041 g) is attributed to the greater incorporation of oleoresin. However, the unchanged thickness (0.27 mm) compared to the 1% film suggests that the high COR load may have plasticized the matrix or induced phase separation, leading to a heterogeneous structure with reduced compactness. This aligns with observations by Liu *et al.* (2020),[43] who noted that excessive essential oil incorporation altered the microstructure of chitosan–gelatin films, leading to thickness reduction due to poor film-forming homogeneity.

Zhang *et al.* (2018)[44] conducted an *in vitro* and *in vivo* study on mucoadhesive films for oral ulcers, finding thicknesses similar to ours (0.3 to 0.6 mm) but slightly higher average weights (44–67 mg for 1 cm^2 discs). Similarly, Averineni *et al.* (2009)[45] developed mucoadhesive films for oral submucous fibrosis using CH and hydroxypropyl methylcellulose (HPMC), where their thickness results denote a thickness higher than that found in the present work, with values exceeding 0.6 mm. Excessive film diameters can cause patient discomfort and interfere with treatment adherence; therefore, thinner films are preferred.

Altogether, these findings highlight how the concentration and distribution of COR within the chitosan matrix govern not only the mechanical but also the dimensional characteristics of the films. Low-to-intermediate COR concentrations (0.5%–1%) favor uniform dispersion and light, flexible films, while the absence of COR (control) or excessive loading (3.5%) increases weight through distinct mechanisms: matrix density in the former and mass load in the latter.

3.4.2 Moisture. Wound dressings should protect and maintain hydration.[46] Moisture determination is crucial, as the film must retain and transport moisture at appropriate levels to prevent drying, influencing adhesion, cell growth, and migration mechanisms.[46] In this study, the moisture content of the films ranged from 15.24% to 20.23% (Fig. 2). Statistically, the differences between the groups were significant (p = 0.025), showing a decreasing trend as the concentration of copaiba oleoresin (COR) increased. These values are consistent with those reported by Paranhos *et al.* (2022),[36] who observed moisture levels between 9% and 21% in chitosan membranes containing copaiba oil.

The reduction in moisture content at higher COR concentrations aligns with previous reports involving essential oils incorporated into chitosan-based matrices.[36,47,48] This behavior is attributed to the hydrophobic nature of oleoresins, which limits hydrogen bonding between water and the polar groups of chitosan. However, it is worth noting that the control film—composed exclusively of chitosan—presented lower moisture content than the films containing 0.5% and 1% COR. This unexpected behavior may be explained by the partial dispersion of COR droplets at low concentrations, which, in the presence of Tween 80, could have facilitated the formation of a more open and flexible polymeric network, enhancing water retention capacity.[47] In contrast, the control film likely formed a denser matrix, with fewer voids and limited free volume for water accommodation, consistent with the findings of Sutharsan *et al.* (2022),[42] who also observed higher moisture content in pure chitosan films due to its hygroscopic nature.

Although Tween 80 was included to aid in the dispersion of hydrophobic compounds, its low concentration was probably insufficient to counteract the barrier effect imposed by higher COR levels. These results highlight the dual role of COR in modulating both moisture retention and water barrier properties of CH-based films. Thus, COR concentration must be optimized to maintain proper hydration while preserving the mechanical and functional performance.

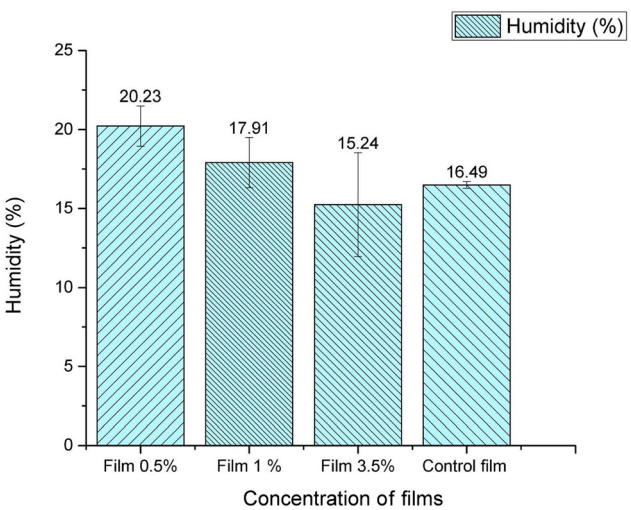

Fig. 2 Moisture percentage of the films at different concentrations of copaiba oleoresin (COR): 0.5%, 1%, 3.5%, and 0% (control).

3.4.3 Swelling degree. The film's ability to absorb fluids and increase its volume is essential for mucoadhesiveness, as it facilitates interaction with the mucosal surface, increasing the contact area and adhesion.[46] However, excessive swelling can compromise the film's structural integrity, reduce adhesion strength, and potentially cause discomfort to the user.[36,46] The swelling results (Fig. 3) revealed a marked water absorption capacity for all tested films, especially during the first hours of immersion in artificial saliva. The data were analyzed considering intra-group comparisons (over time within each concentration) and inter-group comparisons (between different concentrations at each time point). The Friedman test, used for intra-group comparisons, revealed statistically significant differences over time for the control group (0%) (χ^2 = 14.6, p = 0.012), 1% (χ^2 = 11.5, p = 0.043), and 3.5% (χ^2 = 13.9, p = 0.017). Only the 0.5% group showed no significant differences between time points (χ^2 = 7.38, p = 0.194), which may indicate greater swelling stability over the 24-hour period. In the control group, the swelling degree peaked between 4 and 6 hours, with a significant decrease observed after 8 hours, reaching minimum values at 24 hours. The same pattern was observed for the 1% and 3.5% groups, though with distinct magnitudes. This decline suggests a relevant transition point for dressing replacement, around 8 to 10 hours, especially in clinical scenarios involving oral applications. Regarding inter-group analysis, the Kruskal–Wallis test indicated that although no statistically significant differences were observed between concentrations at most time points, a significant difference was detected at the 24-hour mark (χ^2 = 8.87, p = 0.031).

According to Khan *et al.* (2012),[49] the water retention capacity of a film is primarily dependent on the composition of its constituents. The swelling of chitosan-based films is attributed to the presence of amino groups (–NH), which promote hydrogen bonding with water molecules.[5] In addition, the hydrophilic nature of chitosan, combined with plasticizers like glycerin, likely contributed to the high swelling values observed.

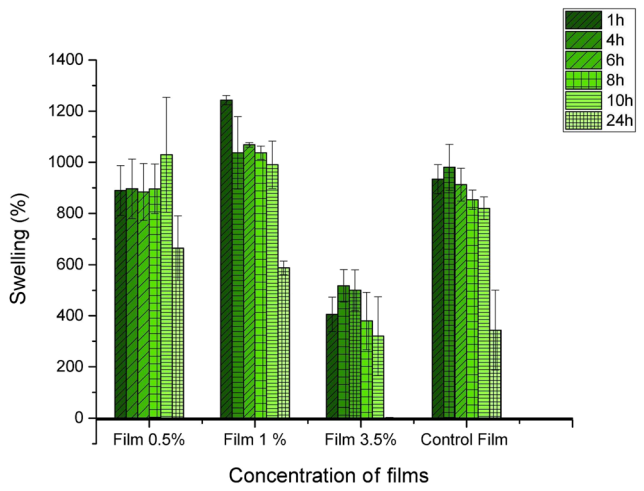

Fig. 3 Swelling percentage of the CH–COR films at different concentrations of copaiba oleoresin (COR): 0.5%, 1%, 3.5%, and 0% (control) over time (1 h, 4 h, 6 h, 8 h, 10 h, and 24 h).

Despite this, excessive water absorption can compromise retention at the application site, potentially making the film slippery—particularly at lower concentrations of COR. Notably, the film containing 3.5% COR exhibited the lowest swelling percentage, consistent with the findings of Paranhos *et al.* (2022),[36] who reported reduced swelling at higher COR concentrations. This behavior may be explained by a reduction in the availability of amino groups due to interactions with the oleoresin and by the hydrophobic nature of COR, which limits water penetration into the matrix.

In this study, the film with 1% COR presented the highest swelling degree, while the control film exhibited the lowest. This trend may be associated with differences in the polymer network structure: at intermediate COR concentrations, the presence of oleoresin may have disrupted the chitosan matrix in a way that created a more porous structure, allowing greater fluid absorption. In contrast, the control film—composed exclusively of chitosan—likely formed a denser and more compact matrix, reducing the availability of free volume for water uptake. Similar behavior was described by Sutharsan *et al.* (2022),[42] who observed that the incorporation of hydrophobic compounds into chitosan films decreased their swelling capacity due to reduced porosity and fewer functional groups available for interaction with water.

Multiple factors may influence swelling, including the inherent hydrophilicity of chitosan, as well as environmental variables such as pH and temperature. More acidic pH levels and higher temperatures are known to enhance the swelling of chitosan-based materials, which could help explain the elevated swelling rates observed in this study.[36]

3.4.4 Solubility degree. Solubility refers to the film's ability to dissolve in the surrounding medium, which is essential for ensuring controlled and sustained drug release. As with swelling, solubility is also a crucial property that directly influences the effectiveness of mucoadhesive films. Films with inadequate solubility may release the drug too quickly or leave residues after application.[40,44] Therefore, a balance between swelling and solubility must be carefully achieved. In this study, the solubility of the films ranged from 94.1% to 96.5% (Fig. 4), and the differences were not statistically significant (p = 0.183), indicating high solubility across all formulations. While this property is generally desirable, excessive solubility can accelerate the release of bioactive compounds, reducing their residence time at the site of application and potentially compromising therapeutic efficacy. Similar trends were reported by Silva (2024),[50] where increased incorporation of *Coffea arabica* L. leaf extracts led to higher solubility values. Likewise, Khoshgozaran-Abras *et al.* (2012)[51] found that solubility in chitosan-based films increased proportionally with the concentration of aloe vera oil.

The greater solubility observed in COR-containing films, particularly at 0.5% and 1%, compared to the control, can be attributed to structural differences in the polymeric matrix.

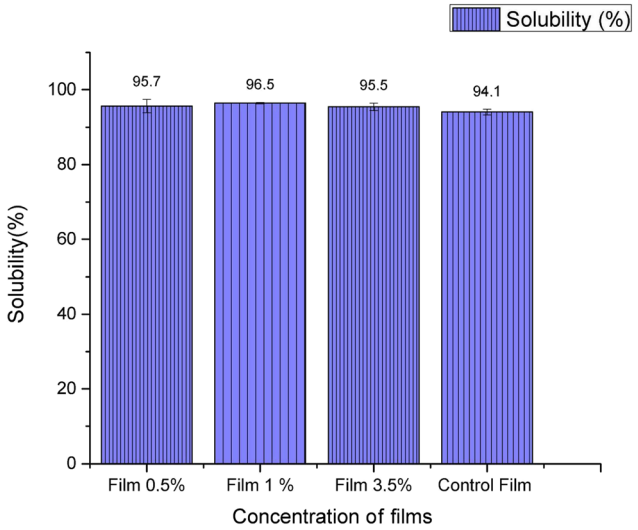

Fig. 4 Solubility percentage of the films at different concentrations of copaiba oleoresin (COR): 0.5%, 1%, 3.5%, and 0% (control).

The control film—composed exclusively of chitosan—likely formed a denser and more compact network, restricting water ingress and thus limiting solubilization. In contrast, the addition of copaiba oleoresin may have disrupted this compactness, generating a more porous matrix that facilitated water diffusion. Additionally, the presence of Tween 80, even at low concentrations, may have improved COR dispersion and increased the surface area available for water interaction, further enhancing solubility.

These findings are in line with those of Peng, Yin, and Li (2012),[48] who reported that essential oils and surfactants can increase film solubility by weakening polymer–lipid interactions and promoting the migration of oil droplets to the surface. Similarly, Sutharsan *et al.* (2022)[42] demonstrated that the incorporation of hydrophobic compounds like epoxy-activated agarose into chitosan films reduced solubility, underscoring the influence of matrix compactness and hydrophobicity on water accessibility.

In our study, a correlation was also observed between solubility and moisture content: films with higher COR concentrations retained more moisture, suggesting increased matrix permeability to water, which in turn favors solubilization. The control film, with lower moisture content and a more cohesive matrix, exhibited the lowest solubility values.

Taken together, the results of solubility, swelling, and moisture content provide an integrated understanding of the physicochemical behavior of CH–COR films. These properties must be carefully modulated to ensure proper hydration, mechanical stability, and effective drug release in biomedical applications.

3.5 Vibrational electronic Raman spectroscopy and Fourier transform infrared (FT-IR) spectroscopy

The spectra of the films and their constituent analysis (Fig. 5A and B, respectively) revealed a predominance of signals related

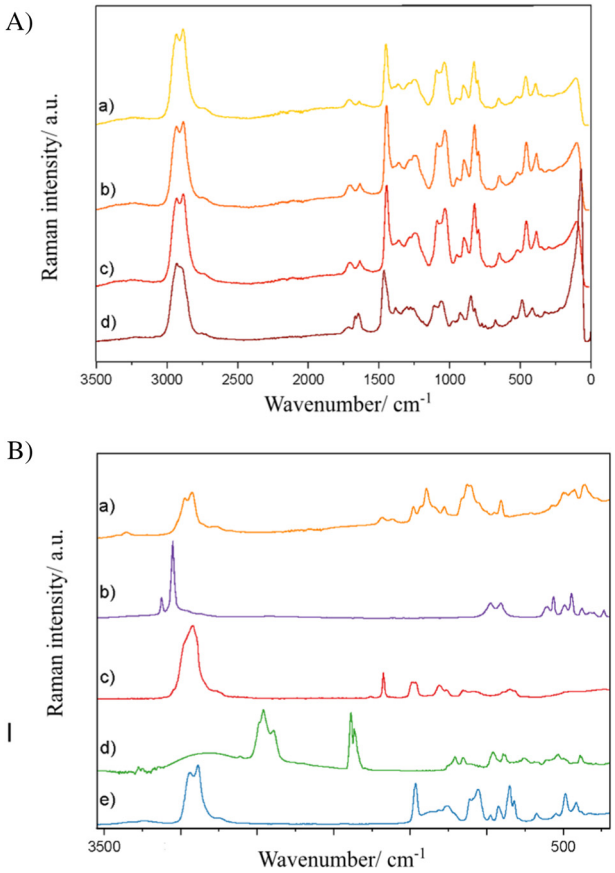

Fig. 5 Raman spectra of CH–COR films and their constituents. (A) Films prepared with different COR concentrations: (a) 0% (control), (b) 0.5%, (c) 1%, and (d) 3.5%. (B) Individual components: (a) chitosan, (b) malic acid, (c) Tween 80, (d) copaiba oleoresin, and (e) glycerin.

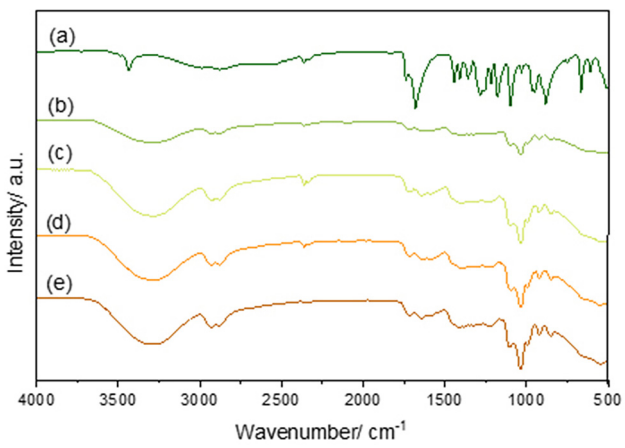

Fig. 6 FT-IR spectra of (a) Malic acid and CH–COR films at different COR concentrations: b) blank film, (c) 0.5% film; d) 1% film; and (e) 3.5% film.

to glycerin, with fewer elements associated with chitosan and Tween 80. However, signals in the region between 1750 and 1500 cm^{-1} can be linked to malic acid. A similar behavior is observed in the FT-IR spectrum from 1750 to 500 cm^{-1} (Fig. 6).

The consistency of the bands in this region suggests that malic acid is present and interacts with other components of the film matrix. Moreover, the intensity and shape of the spectral signals may indicate molecular interactions between malic acid and other film constituents, such as chitosan or copaiba oleoresin. This suggests that, although the concentration of malic acid has not varied, its interaction with the polymers or the way it organizes within the matrix may influence the detected spectral behavior. These interactions are evident in the subtle changes observed in the spectral patterns among the different samples.

The presence of chitosan is indicated by the N–H and O–H stretching bands, which usually appear between 3400 and 3200 cm^{-1}, in addition to the C–O–C and C–N stretching vibrations around 1150–1020 cm^{-1}.[52,53] The predominant signals of glycerin can be observed in the 2900–2800 cm^{-1} regions, which correspond to C–H stretching vibrations, and in the 1000–800 cm^{-1} range, associated with CH_2 twisting vibrations.[54] The bands in the 1750–1500 cm^{-1} region are associated with C=O[52] stretching present in malic acid.

The spectroscopic analysis indicates the presence and integration of the film components, highlighting the molecular interactions between malic acid, chitosan, and copaiba oleoresin. The identification of characteristic signals from each component, such as the N–H and O–H vibrations of chitosan and the bands associated with malic acid, suggests a homogeneous distribution of the constituents within the matrix.

3.6 Scanning electron microscopy (SEM)

SEM analysis of the film surfaces revealed few irregularities across all tested concentrations, including the control (Fig. 7). Small irregularities were visible at the film edges, particularly where the samples were cut. In films with added COR, microscopy highlighted small droplets dispersed in the matrix, especially in films with higher COR concentrations. This contrasts with the findings of Debone *et al.* (2019),[55] who, despite not observing macroscopic phase separation, noted heterogeneous droplet distribution in chitosan film micrographs. Their findings indicated that COR/chitosan films showed a discontinuous pattern associated with a lipid phase dispersed in the polymer matrix, while oil-free films presented a more homogeneous microstructure without discontinuities, and the size of oil droplets increased with higher COR concentrations, which was not observed in the present study. Debone *et al.* (2019)[55] did not describe the use of surfactants in the formulation, which may have influenced COR dispersion in the polymer matrix, highlighting the importance of Tween 80 in the formulation. A similar behavior is observed in Paranhos *et al.* (2022),[36] with noticeable copaiba oil droplets in the chitosan matrix, where no surfactant use was mentioned. Unlike previous studies that reported heterogeneous droplet distribution, the use of Tween 80 in the present formulation appears to have contributed to a more consistent dispersion of COR, preventing phase separation. These findings emphasize the importance of surfactants in ensuring homogeneity within polymeric films.

3.7 Biological evaluation of CH–COR films

3.7.1 Cell viability assay. According to the International Standard ISO 10993-5,[56] a material is considered cytotoxic when cell viability is less than 70% compared to untreated control cells. The cytotoxicity of CH–COR films and their components was assessed using the MTT assay. However, reliable cytotoxic activity analysis was not possible due to the difficulty in removing the films from the cell culture wells. The physical presence of the films likely created a barrier, preventing direct contact between cells and assay reagents. This barrier may have interfered with cell viability by limiting the diffusion of nutrients and reagents, leading to misleading results. Therefore, the apparent cytotoxicity observed may not reflect the true biocompatibility of the films but rather a false positive effect due to their physical interaction with the cells. Results for COR and CH are presented in Fig. 8. COR and its isolated dilutions were not cytotoxic and even promoted the growth of J774 cells. Our results confirm the safety of chitosan on the tested cells, reinforcing its recognized biocompatibility. Chitosan is well-documented in the scientific literature for its low toxicity.[3–6] The low toxicity observed in the tests further

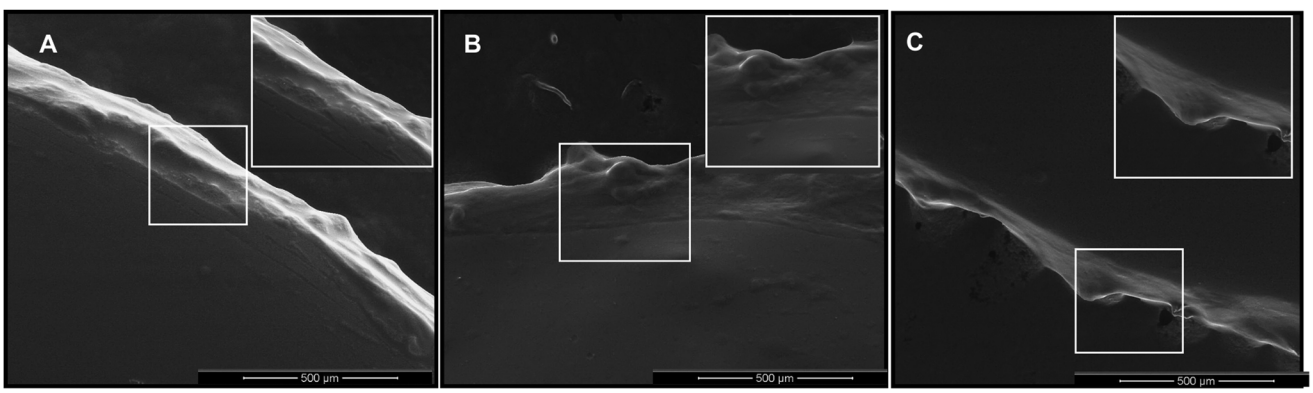

Fig. 7 Scanning electron microscopy of the films at different concentrations, with magnifications of 200× and 500×. A: Control film; B: 0.5% film; and C: 1% film.

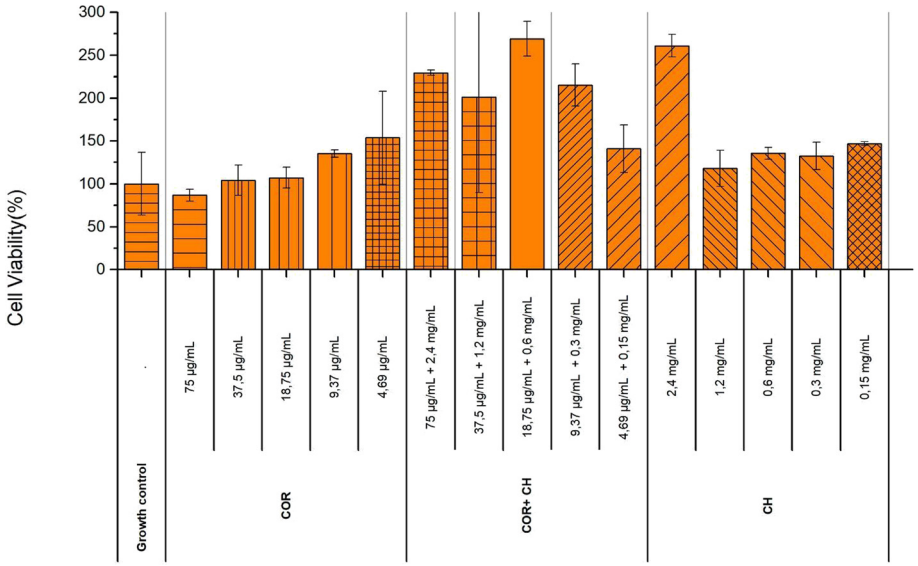

Fig. 8 Cell viability in relation to the concentrations of COR, CH, and COR + CH present in the films.

supports its potential as a safe and effective component in therapeutic formulations. According to Genesi *et al.* (2023),[18] who used Balb/c 3T3 clone A31 cells to test the cytotoxicity of chitosan films incorporated with aloe vera and copaiba oil, results indicated that the tested films exceeded the ISO 10993-5 limit and were considered cytotoxic, except for films with 0.5% aloe vera and 0.5% copaiba oil, which showed increased cell viability, suggesting a need for further investigation into the effects of these components on cultured cells. Conversely, Sun *et al.* (2012)[57] studied chitosan films containing hydroxyapatite and assessed the cytotoxicity of the composites in L-929 fibroblasts. They observed that cells exposed to different concentrations of the diluted film extracts did not show a significant reduction in viability, indicating the biocompatibility of the composite films. These results suggest that COR and CH are promising and safe agents. However, further research and testing are necessary to improve analysis methods and more accurately evaluate the cytotoxicity of the films. Considering that the ultimate goal of these films is the treatment of oral mucositis, it is essential to ensure that they are not only effective but also safe and comfortable for patients.

3.7.2 Antimicrobial activity. The evaluation of antimicrobial activity often uses the agar diffusion method due to its simplicity and low cost. This method has two main variations: disk diffusion and well diffusion. Disk diffusion is the most commonly used in the literature and was chosen for this study. However, its sensitivity is not comparable to that of the Minimum Inhibitory Concentration (MIC) test, as various factors can influence and hinder compound diffusion in the agar (Scorzoni, L. *et al.*, 2007),[58] thereby affecting the inhibition zone and result interpretation.

In the present study, the results did not establish a clear relationship between the concentrations and strains tested, which might be due to the lack of uniform diffusion of the active compounds in the agar and the film absorbing moisture from the culture medium and swelling, complicating the interpretation and discussion of the results. In comparison, the work of Gomes *et al.* (2024)[11] focused on the antimicrobial potential of *C. officinalis* oleoresin and chitosan through the MIC assay, bypassing the challenges posed by agar diffusion. Their study demonstrated that both copaiba oleoresin and chitosan exhibit significant antimicrobial properties against oral pathogens. These findings highlight that, although the current study encountered challenges with the agar diffusion method, the antimicrobial potential of the same active ingredients—copaiba oleoresin and chitosan—has been demonstrated in other experimental setups. This suggests that the difficulties observed in this study may be related more to the limitations of the agar diffusion method than to the inherent antimicrobial potential of the films. According to Genesi *et al.* (2023),[18] there seems to be an incompatibility between the Mueller–Hinton culture medium and the components present in the film, which further complicates the interpretation of the antimicrobial activity. Pereda *et al.* (2011)[52] also emphasized that the physical properties of biofilms can hinder their diffusion in agar.

4. Conclusion

The CH–COR film developed in this study exhibited promising chemical, physical, and biological characteristics. Spectroscopic and SEM analyses demonstrated relevant molecular interactions between the components. Although the formulation presented high water absorption and solubility, cytotoxicity assessment was limited by the methodology employed.

Importantly, none of the tested concentrations exhibited universally superior performance across all evaluated para-

meters. While the 1% COR film demonstrated higher swelling and moisture retention—potentially enhancing flexibility and hydration—this may compromise mucoadhesion due to overhydration. At the 3.5% COR film showed reduced swelling and moisture content, which may improve mucosal retention, but its increased weight and potential heterogeneity could affect patient comfort. The 0.5% COR film exhibited intermediate characteristics, suggesting a more balanced profile, although not necessarily optimal.

Therefore, no single formulation can currently be recommended as the most appropriate based solely on the results obtained. The choice of concentration should be guided by the specific therapeutic objectives, such as immediate bioactive release *versus* prolonged mucosal residence.

As this is a preliminary study, the release profile of β-caryophyllene was not assessed. This decision was based on the primary aim of evaluating the feasibility of incorporating copaiba oleoresin into chitosan films and conducting initial physicochemical and antimicrobial characterization studies. However, further studies are warranted to investigate the release kinetics of the active compound under simulated oral conditions, which will be essential to correlate bioactive diffusion with therapeutic activity.

Additionally, although mucoadhesion is a critical feature for oral dressings, no specific adhesive strength or mucosal retention time tests were performed in this study. These analyses will be essential in future investigations to better assess the clinical applicability of the films and ensure their effective residence at the application site.

Future investigations should explore the mucoadhesive performance, long-term stability, thermal behavior, and *in vivo* biocompatibility of the films. These developments will support the rational design of multifunctional delivery systems tailored for clinical use in oral mucosal repair.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Due to the patented nature of the formulation, some restrictions may apply to the availability of proprietary information.

Acknowledgements

The authors gratefully acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarships provided. and the Federal University of Juiz de Fora (UFJF/Brazil) for the scholarships provided.

References

1 S. Elad, N. Yarom, Y. Zadik, M. Kuten-Shorrer and S. T. Sonis, The broadening scope of oral mucositis and oral ulcerative mucosal toxicities of anticancer therapies, *CA-Cancer J. Clin.*, 2022, **72**(1), 57–77.
2 Z. Zhang, Q. Zhang, S. Gao, H. Xu, J. Guo and F. Yan, Antibacterial, anti-inflammatory and wet-adhesive poly (ionic liquid)-based oral patch for the treatment of oral ulcers with bacterial infection, *Acta Biomater.*, 2023, **166**, 254–265.
3 M. E. Abd El-Hack, M. T. El-Saadony, M. E. Shafi, N. M. Zabermawi, M. Arif, G. E. Batiha, *et al.*, Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review, *Int. J. Biol. Macromol.*, 2020, **164**, 2726–2744.
4 W. Wang, Q. Meng, Q. Li, J. Liu, M. Zhou, Z. Jin, *et al.*, Chitosan Derivatives and Their Application in Biomedicine, *Int. J. Mol. Sci.*, 2020, **21**(2), 487.
5 Y. Xia, D. Wang, D. Liu, J. Su, Y. Jin, D. Wang, *et al.*, Applications of Chitosan and its Derivatives in Skin and Soft Tissue Diseases, *Front. Bioeng. Biotechnol.*, 2022, **10**, 894667.
6 S. Petroni, I. Tagliaro, C. Antonini, M. D'Arienzo, S. F. Orsini, J. F. Mano, *et al.*, Chitosan-Based Biomaterials: Insights into Chemistry, Properties, Devices, and Their Biomedical Applications, *Mar. Drugs*, 2023, **21**(3), 147–147.
7 J. J. de Lima Silva, S. B. Guimarães, E. R. da Silveira, P. R. de Vasconcelos, G. G. Lima, S. M. Torres and R. C. de Vasconcelos, Effects of Copaifera langsdorffii Desf. on Ischemia-Reperfusion of Randomized Skin Flaps in Rats, *Aesthetic Plast. Surg.*, 2009, **33**(1), 104–109.
8 F. B. Teixeira, R. de Brito Silva, O. A. Lameira, L. P. Webber, R. S. D'Almeida Couto, M. D. Martins, *et al.*, Copaiba oil-resin (Copaifera reticulata Ducke) modulates the inflammation in a model of injury to rats' tongues, *BMC Complementary Altern. Med.*, 2017, **17**(1), 313.
9 D. Bardaji, J. Gomes, T. C. Bianchi, F. de Daniele, L. F. Leandro, *et al.*, Copaifera reticulata oleoresin: Chemical characterization and antibacterial properties against oral pathogens, *Anaerobe*, 2016, **40**, 18–27.
10 A. L. Diefenbach, F. W. M. G. Muniz, H. J. R. Oballe and C. K. Rösing, Antimicrobial activity of copaiba oil (Copaifera ssp.) on oral pathogens: Systematic review, *Phytother. Res.*, 2017, **32**(4), 586–596.
11 T. V. Gomes, M. Simões, A. B. Polo, B. V. Tomé, M. M. Cardoso, G. D. Tavares, *et al.*, Combined potential of copaifera officinalis oleoresin and chitosan against oral pathogens, *Braz. J. Microbiol.*, 2024, 3973–3978.
12 I. Bano, M. Arshad, T. Yasin, M. A. Ghauri and M. Younus, Chitosan: A potential biopolymer for wound management, *Int. J. Biol. Macromol.*, 2017, **102**, 380–383.

13 F. S. Rezaei, F. Sharifianjazi, A. Esmaeilkhanian and E. Salehi, Chitosan films and scaffolds for regenerative medicine applications: A review, *Carbohydr. Polym.*, 2021, **273**, 118631. Available from: https://www.sciencedirect.com/science/article/pii/S0144861721010183?casa_token=7Bvz2B07HZkAAAAA:ZwKV6TgI2ZBWgk5Vqp_pQ9qUiOhzOwJ0U1JhcxX5kaLMH4XjzAZFfq_FgluYaXrnEu_pkE_#bbb0465.

14 I. A. Sogias, V. V. Khutoryanskiy and A. C. Williams, Exploring the Factors Affecting the Solubility of Chitosan in Water, *Macromol. Chem. Phys.*, 2009, **211**(4), 426–433.

15 S. Watanabe, K. Suemaru, T. Yamaguchi, N. Hidaka, M. Sakanaka and H. Araki, Effect of oral mucosal adhesive films containing ginsenoside Rb1 on 5-fluorouracil-induced oral mucositis in hamsters, *Eur. J. Pharmacol.*, 2009, **616**(1–3), 281–286.

16 S. Watanabe, K. Suemaru, K. Takechi, H. Kaji, K. Imai and H. Araki, Oral mucosal adhesive films containing royal jelly accelerate recovery from 5-fluorouracil-induced oral mucositis, *J. Pharmacol. Sci.*, 2013, **121**(2), 110–118 [cited 2021 Dec 9]. Available from: https://pubmed.ncbi.nlm.nih.gov/23357874/.

17 H. S. Debone, P. S. Lopes, P. Severino, C. M. P. Yoshida, E. B. Souto and C. F. da Silva, Chitosan/Copaiba oleoresin films for would dressing application, *Int. J. Pharm.*, 2019, **555**, 146–152.

18 B. P. Genesi, R. M. Barbosa, P. Severino, A. C. D. Rodas, C. M. P. Yoshida, M. B. Mathor, *et al.*, Aloe vera and copaiba oleoresin-loaded chitosan films for wound dressings: microbial permeation, cytotoxicity, and in vivo proof of concept, *Int. J. Pharm.*, 2023, **634**, 122648–122648.

19 P. Aksungur, A. Sungur, S. Ünal, A. B. İskit, C. A. Squier and S. Şenel, Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies, *J. Controlled Release*, 2004, **98**(2), 269–279.

20 R. P. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry*, Allured Business Media, Carol Stream, Illinois, 2012.

21 H. van Den Dool and P. D. Kratz, A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography, *J. Chromatogr., A*, 1963, **11**, 463–471. Available from: https://www.sciencedirect.com/science/article/abs/pii/S002196730180947X.

22 P. Cazón, A. Antoniewska, J. Rutkowska and M. Vázquez, Evaluation of easy-removing antioxidant films of chitosan with Melaleuca alternifolia essential oil, *Int. J. Biol. Macromol.*, 2021, **186**, 365–376.

23 A. Shah, M. Ali Buabeid, E. S. A. Arafa, I. Hussain, L. Li and G. Murtaza, The wound healing and antibacterial potential of triple-component nanocomposite (chitosan-silver-sericin) films loaded with moxifloxacin, *Int. J. Pharm.*, 2019, **564**, 22–38.

24 N. Devi and J. Dutta, Preparation and characterization of chitosan-bentonite nanocomposite films for wound healing application, *Int. J. Biol. Macromol.*, 2017, **104**, 1897–1904.

25 Agência Nacional De Vigilância Sanitária (Brazil, Fundação Oswaldo Cruz. Farmacopeia brasileira. Brasília: Agência Nacional De Vigilância Sanitária, 2010.

26 C. Pansara, R. Mishra, T. Mehta, A. Parikh and S. Garg, Formulation of chitosan stabilized silver nanoparticle-containing wound healing film: *In vitro* and *in vivo* characterization, *J. Pharm. Sci.*, 2020, 2196–2205.

27 A. W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turck, Antibiotic Susceptibility Testing by a Standardized Single Disk Method, *Am. J. Clin. Pathol.*, 1966, **45**(4_ts), 493–496.

28 J. Lee, M. Wang, J. Zhao, Z. Ali, M. F. Hawwal and I. A. Khan, Chemical Characterization and Quality Assessment of Copaiba Oil-Resin Using GC/MS and SFC/MS, *Plants*, 2023, **12**(8), 1619–1619.

29 A. P. Ames-Sibin, C. L. Barizão, C. V. Castro-Ghizoni, F. M. S. Silva, A. B. Sá-Nakanishi, L. Bracht, *et al.*, β-Caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats, *J. Cell. Biochem.*, 2018, **119**(12), 10262–10277.

30 L. M. Leandro, F. de Sousa Vargas, P. C. S. Barbosa, J. K. O. Neves, J. A. da Silva and V. F. da Veiga-Junior, Chemistry and Biological Activities of Terpenoids from Copaiba (Copaifera spp.) Oleoresins, *Molecules*, 2012, **17**(4), 3866–3889. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6269112/.

31 F. Gelmini, G. Beretta, C. Anselmi, M. Centini, P. Magni, M. Ruscica, *et al.*, GC–MS profiling of the phytochemical constituents of the oleoresin from Copaifera langsdorffii Desf. and a preliminary in vivo evaluation of its antipsoriatic effect, *Int. J. Pharm.*, 2013, **440**(2), 170–178. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0378517312008162?via%3Dihub.

32 Y. Chu, T. Xu, C. Gao, X. Liu, N. Zhang, X. Feng, *et al.*, Evaluations of physicochemical and biological properties of pullulan-based films incorporated with cinnamon essential oil and Tween 80, *Int. J. Biol. Macromol.*, 2019, **122**, 388–394 [cited 2022 Oct 25]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0141813018336821.

33 A. G. Darbasi, H. Kiani and F. Khodaiyan, Development of chitosan based extended-release antioxidant films by control of fabrication variables, *Int. J. Biol. Macromol.*, 2017, **104**, 303–310.

34 G. Biliuta, S. P. Gherman, R. I. Baron, A. Bargan, L. Ochiuz, C. G. Tuchilus, *et al.*, Development and Characterization of Polymeric Films Loaded with Terbinafine for Fungal Infection Treatment, *Polymers*, 2025, **17**(8), 1004–1004.

35 K. Tenorová, R. Masteiková, S. Pavloková, K. Kostelanská, J. Bernatoniene and D. Vetchý, Formulation and Evaluation of Novel Film Wound Dressing Based on Collagen/Microfibrillated Carboxymethylcellulose Blend, *Pharmaceutics*, 2022, **14**(4), 782–782. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9027540/.

36 S. B. Paranhos, E. da S. Ferreira, C. A. de A. Canelas, S. P. A. da Paz, M. F. Passos, C. E. F. da Costa, *et al.*, Chitosan Membrane Containing Copaiba Oil (Copaifera spp.) for Skin Wound Treatment, *Polymers*, 2021, **14**(1), 35,

[cited 2022 Oct 19]. Available from: https://pubmed.ncbi.nlm.nih.gov/35012060/.

37 W. G. Sganzerla, L. E. N. Castro, C. G. Rosa, A. R. Almeida, F. W. Maciel-Silva, *et al.*, Production of nanocomposite films functionalized with silver nanoparticles bioreduced with rosemary (Rosmarinus officinalis L.) essential oil, *J. Agric. Food Res.*, 2023, **11**, 100479–100479.

38 B. Heurtault, Physico-chemical stability of colloidal lipid particles, *Biomaterials*, 2003, **24**(23), 4283–4300.

39 F. G. de Carvalho, T. C. Magalhães, N. M. Teixeira, B. L. C. Gondim, H. L. Carlo, R. L. dos Santos, *et al.*, Synthesis and characterization of TPP/chitosan nanoparticles: Colloidal mechanism of reaction and antifungal effect on C. albicans biofilm formation, *Mater. Sci. Eng., C*, 2019, **104**, 109885, [cited 2023 Jun 30]. Available from: https://www.sciencedirect.com/science/article/pii/S0928493118331345.

40 S. Suzuki, B. Ying, H. Yamane, H. Tachi, K. Shimahashi, K. Ogawa, *et al.*, Surface structure of chitosan and hybrid chitosan-amylose films—restoration of the antibacterial properties of chitosan in the amylose film, *Carbohydr. Res.*, 2007, **342**(16), 2490–2493.

41 I. S. M. Costa, R. P. Abranches, M. T. J. Garcia and M. B. R. Pierre, Chitosan-based mucoadhesive films containing 5-aminolevulinic acid for buccal cancer's treatment, *J. Photochem. Photobiol., B*, 2014, **140**, 266–275.

42 J. Sutharsan, C. A. Boyer and J. Zhao, Development and characterization of chitosan edible film incorporated with epoxy–activated agarose, *JSFA Rep.*, 2022, **2**(12), 613–622.

43 T. Liu, J. Wang, F. Chi, Z. Tan and L. Liu, Development and Characterization of Novel Active Chitosan Films Containing Fennel and Peppermint Essential Oils, *Coatings*, 2020, **10**(10), 936.

44 C. Zhang, Y. Liu, W. Li, P. Gao, D. Xiang, X. Ren, *et al.*, Mucoadhesive buccal film containing ornidazole and dexamethasone for oral ulcers: in vitro and in vivo studies, *Pharm. Dev. Technol.*, 2018, **24**(1), 118–126.

45 R. K. Averineni, S. G. Sunderajan, S. Mutalik, U. Nayak, G. Shavi, K. Armugam, *et al.*, Development of mucoadhesive buccal films for the treatment of oral sub-mucous fibrosis: a preliminary study, *Pharm. Dev. Technol.*, 2009, **14**(2), 199–207.

46 K. Nuutila and E. Eriksson, Moist Wound Healing with Commonly Available Dressings, *Adv. Wound Care*, 2020, (12), 10.

47 L. Sánchez-González, M. Cháfer, A. Chiralt and C. González-Martínez, Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on Penicillium italicum, *Carbohydr. Polym.*, 2010, **82**(2), 277–283.

48 Y. Peng, L. Yin and Y. Li, Combined effects of lemon essential oil and surfactants on physical and structural properties of chitosan films, *Int. J. Food Sci. Technol.*, 2012, **48**(1), 44–50, [cited 2025 Mar 11]. Available from: https://academic.oup.com/ijfst/article/48/1/44/7865447.

49 A. Khan, R. A. Khan, S. Salmieri, C. Le Tien, B. Riedl, J. Bouchard, *et al.*, Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films, *Carbohydr. Polym.*, 2012, **90**(4), 1601–1608. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0144861712006935.

50 A. Silva, A. Beatriz, A. Sérgio de Oliveira Lemos, L. M. Campos, R. L. Fabri, F. F. Costa, *et al.*, Development and characterization of chitosan film containing hydroethanolic extract of Coffea arabica leaves for wound dressing application, *Mater. Today Commun.*, 2024, **38**, 108503–108503.

51 S. Khoshgozaran-Abras, M. H. Azizi, Z. Hamidy and N. Bagheripoor-Fallah, Mechanical, physicochemical and color properties of chitosan based-films as a function of Aloe vera gel incorporation, *Carbohydr. Polym.*, 2012, **87**(3), 2058–2062.

52 M. Pereda, A. G. Ponce, N. E. Marcovich, R. A. Ruseckaite and J. F. Martucci, Chitosan-gelatin composites and bilayer films with potential antimicrobial activity, *Food Hydrocolloids*, 2011, **25**(5), 1372–1381.

53 E. Vieira, A. R. Cestari, R. A. Chagas and K. Cortes, Development and characterization of a matrix suitable for extended release systems - release studies with the herbicides atrazine and diuron, *Quim. Nova*, 2014, **37**(3), DOI: 10.5935/0100-4042.20140074.

54 P. Wong, Raman Spectroscopy of Thermotropic and High-Pressure Phases of Aqueous Phospholipid Dispersions, *Annu. Rev. Biophys. Biomol. Struct.*, 1984, **13**(1), 1–24.

55 H. S. Debone, P. S. Lopes, P. Severino, C. M. P. Yoshida, E. B. Souto and C. F. da Silva, Chitosan/Copaiba oleoresin films for would dressing application, *Int. J. Pharm.*, 2019, **555**, 146–152.

56 . ISO 10993-5:2009. Biological evaluation of medical devices—part 5: tests for in vitro cytotoxicity. International Organization for Standardization, 2009.

57 F. Sun, K. Koh, S. C. Ryu, D. W. Han and J. Lee, Biocompatibility of Nanoscale Hydroxyapatite-embedded Chitosan Films, *Bull. Korean Chem. Soc.*, 2012, **33**(12), 3950–3956.

58 L. Scorzoni, T. Benaducci, A. M. F. Almeida, D. Helena, S. Bolzani and M. José, Comparative study of disk diffusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts Candida spp and Cryptococcus sp, *Dir. Open Access J.*, 2007, 25–34.