Journal of Composites and Compounds 6(2024) 1-5

Available Online at www.jourcc.com

Journal homepage: www.JOURCC.com

Journal of Composites and Compounds

Probabilistic modeling of mechanical properties in thymol-loaded gelatin films for nano wound dressing applications

Nadia Banitorfi Hoveizavi [a]*, Mastafa H. Al-Musawi [b]*

[a] *Department of Textile Engineering, Isfahan University of Technology, P. O. Box: 84156-83111, Isfahan, Iran*

[b] *Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq*

A B S T R A C T

Gelatin-based films modified to contain thymol represent an exciting new bioactive material class designed for advanced wound dressing applications. Thymol provides favorable antioxidant and antimicrobial activity; however, we need a quantitative understanding of its effect on the ability of gelatin films to bear a load, which will enable the rational design of these products. The goal of this work was to develop a physics-informed probabilistic modeling framework to predict and analyze the mechanical properties (tensile strength (TS), elongation at break (EAB), and Young's modulus (E)) of thymol-loaded gelatin films as a function of thymol concentration (0–8% w/w), using published experimental data. We fit exponential decay and saturating functions to capture the concentration dependent trends, and used a Monte Carlo simulation (5,000 trials) to quantify the uncertainty in the parameters and to estimate the probability of meeting functional mechanical thresholds (TS > 1.5 N/m^2, EAB > 155%, E > 2.0 MPa). Our work shows a fundamental trade-off effect: thymol provides an increase in flexibility (EAB), yet at the same time diminishes the strength and stiffness. The best range of thymol for the balance of performance is 2-4%, at which the likelihood of meeting all three criteria is highest. This work offers a solid data-backed foundation to modify the mechanical characteristics of bioactive gelatin films, making it easier to create an effective nano wound dressing with antimicrobial properties and structural stability.

A R T I C L E   I N F O R M A T I O N

Article History:
Received 25 June 2024
Received in revised form 16 September 2024
Accepted 20 September 2024

Keywords:
Gelatin-based films
Thymol
Mechanical properties
Modeling
Monte Carlo simulation

1. Introduction

Gelatin, a natural biopolymer, is obtained by controlled hydrolysis of a fibrous insoluble protein called collagen, which is found in an abundance of animal skin and bones. Gelatin is being proposed as a promising biomaterial for a wound dressing because of its natural biocompatibility and biodegradability and capacity to absorb wound exudates while providing a moist wound healing environment [1]. An ideal moist wound dressing should have several functional attributes: to protect against microbial infection; control and absorb wound exudate and blood; keep the wound hydrated; allow gas exchange; protect against thermal injury; and be non-toxic, non-allergenic, comfortable to wear, and easy to remove [2].

Although these beneficial characteristics exist, native gelatin has no inherent antimicrobial activity, restricting its capability to offer protection against wound infection. To enhance this limitation, a variety of antimicrobial agents (for example, silver nanoparticles [3] and zinc oxide nanoparticles [4]), have been added to gelatin matrices. More recently, natural bioactive compounds, especially phenolic monoterpenes from medicinal herbs, are gaining interest as safe and effective candidates for boosts in antimicrobial activity in biopolymer-based dressings.

Thymol (2-isopropyl-5-methylphenol), a major component of essential oils from plants in the genera Origanum, Thymus, Coridothymus, Thymbra, Satureja, and Lippia, has long been used as a natural food preservative [5]. Its broad-spectrum biological activities, including antioxidant [6], antibacterial [7, 8], antifungal [9, 10], and antiparasitic properties [11], are well documented. Nevertheless, the full potential of thymol in advanced biomedical applications, particularly in wound care, remains underexplored.

Mechanical performance is a key consideration for handling, adhesion and durability in clinical settings of gelatin-based wound dressings. Kavoosi and colleagues [12] assessed three important mechanical properties (tensile strength [TS], elongation at break [EAB] and Young's modulus [E]) based on ASTM D638-02a, and

* Corresponding authors: Nadia Banitorfi Hoveizavi & Mastafa H. Al-Musawi, Emails: n.banitorfi@tx.iut.ac.ir, mustafa.h.j@uomustansiriyah.edu.iq

https://doi.org/10.61882/jcc.6.3.6

showed that cross-linking with glutaraldehyde (2%, w/w) improved the mechanical properties of at least a subset of gelatin films. For example, TS was increased from 2.9 N/m^2 (non-cross-linked) to a more robust 4.0 N/m^2; EAB increased from 57% to a more favorable 125%; and Young's modulus was reduced from 10.7 MPA to a softer 8.7 MPA ($P < 0.05$). Thus, cross-linking clearly improves gel strength and flexibility while also reducing stiffness, all attributes desirable in conformable wound dressings.

The addition of thymol (1–8% w/w) to cross-linked films resulted in a significant, concentration-dependent change in mechanical behavior: TS was significantly reduced from 4.0 N/m^2 (0% thymol) to 1.2 N/m^2 (8% thymol); EAB increased from 125% to 170%; and Young's modulus decreased from 8.7 MPa to 1.8 MPa. This behavior is likely a function of thymol's ability to disrupt intermolecular bonding, such as hydrogen bonding and hydrophobic interactions, between gelatin chains, potentially reducing, or interfering with glutaraldehyde-mediated cross-linking as well. Overall, the resulting network has less cohesion (lower TS and E) but greater extensibility (higher EAB), an apparent trade-off also observed in other essential oil–formulations involving biopolymers (e.g., citrus oil in gelatin; [13]). However, while the TS is reduced, the films maintained adequate mechanical strength for use in the lab setting, but particularly at lower concentrations of thymol (1–2%) while attaining increased flexibility to accommodate the dynamic nature of wounds.

To facilitate rational design of such materials, this work provides a computational modeling framework for predicting the mechanical properties tensile strength, elongation at break, and modulus of gelatin films loaded with thymol, as a function of thymol concentration. By combining physics-informed empirical models with Monte Carlo simulations, we quantify uncertainty in model parameters, and we assess the likelihood of meeting mechanical performance targets. Using a probabilistic approach, we identify a desirable thymol concentration range that provides some degree of antimicrobial function while maintaining the mechanical properties necessary for viable nano wound dressing applications.

2. Materials and methods

The mechanical characteristics of wound dressings prepared with biopolymer-based polymers are determined by the interactions of polymer chains and the crosslinking density of the polymer, in combination with the addition of bioactive additives. In the case of gelatin films, the scaffolding within the material has a native network stabilized by weak noncovalent interactions, specifically hydrogen bonding and hydrophobic interactions. Consequently, the material has lower overall mechanical properties. Using crosslinking agents like glutaraldehyde introduces covalent bonds between gelatin chains, which improves mechanical properties. However, the addition of a hydrophobic bioactive like thymol disturbs both the native gelatin network and the glutaraldehyde crosslinking, and this results in an alteration within the mechanical properties of the gelatin film.

In order to quantify these effects we will use physics-informed empirical models that are built off of observations from experimental data [12]. Tensile strength (TS) and Young's modulus (E) both monotonically decrease with increasing thymol concentration. This is in accordance with the loss of cohesive strength within the polymer matrix. This can be modeled using the following exponential decay function:

$$TS(v) = ae^{-bv} + c \quad (1)$$

$$E(v) = a'^{e^{-b'v}} + c' \quad (2)$$

where v is thymol concentration (% w/w), a (a') represents the initial contribution from the cross-linked network, b (b') governs the rate of decay due to thymol-induced disruption, and c (c') is the asymptotic residual strength/stiffness at high thymol loading.

The elongation at break (EAB) increases with thymol concentration, indicative of greater chain mobility and flexibility of the films. This saturating behavior is encapsulated by:

$$EAB = a'' - b''e^{-c''v} \quad (3)$$

where a'' is the maximum achievable elongation, b'' is the initial deficit relative to the saturated state, and c'' controls the rate of approach to the asymptote.

With the limited dataset of the experiment (five data points per property), parameter estimates from the nonlinear regression cannot reflect true uncertainty in estimates. To carefully track uncertainty, we take a residual based Monte Carlo simulation approach:

1. From the initial fit, we calculate the residual standard deviation which offers an estimate for measurement error and model error.
2. A synthetic dataset is created by adding Gaussian noise to the original measurements (scaled by the residual standard deviation).
3. The model is fitted to each synthetic dataset to produce a distribution of estimates of the parameters {a, b, c, a', b', c'. a'', b'', c''}. These parameter ensembles are used to produce prediction bands (95% intervals) and probability maps to evaluate if the measurements exceed user-defined threshold opportunities (e.g. TS > 1.5 N/m2, EAB > 155%, E > 2.0 MPa).

This probabilistic approach allows for sparse experimental measurements to emerge as a design tool that provides useful background risk towards defining optimal thymol concentration to a balance of antimicrobial efficacy and mechanical reliability for nano wound dressing applications. The approach mirrors modern ideas about biomaterials engineering where uncertainty quantification is necessary for operability.

3. Results and discussion

The mechanical behavior of gelatin films loaded with thymol was quantitatively modeled using physics-informed empirical functions fitted to experimental data from [12]. The resulting models predict, with high fidelity, the concentration-dependent evolution of tensile strength (TS), Young's modulus (E), and elongation at break (EAB):

$$TS(v) = 2.73e^{-1.81v} + 1.26 \quad (4)$$

$$E(v) = 6.71^{e^{-2.72v}} + 1.98 \quad (5)$$

$$EAB = 170.268 - 43.84e^{-0.419v} \quad (6)$$

where v is thymol concentration (% w/w). TS and E both showed that, overall, they decline rapidly in an exponential manner as more thymol is added (Fig. 1A, 1C). Knowing that at 0% thymol the model estimated TS to be approximately 4.00 N/m^2 and E ~ 8.70 MPa is an appropriate approximation to the experimental values for cross-linked gelatin films found in the literature [12]. Yet, with just 1% thymol, TS is reduced by almost 50% to ~ 1.7 N/m^2 and ~ 70% to ~ 2.4 MPa. This change may be explained by thymol interfering with cross-linking via glutaraldehyde and acting to disrupt hydrogen bonding and hydrophobic interactions between gelatin chains. The smaller b factor of E, (b=2.720) compared to TS (b=1.801) likely indicates to the stiffness of the film being more sensitive to the incorporation of thymol than ultimate strength, and is consistent with the expected plasticizing behavior of hydrophobic phenolic compounds within biopolymer matrices. In contrast, EAB increases monotonically with thymol concentration, from ≈126% at 0% thymol to ≈170% at 8% (Fig. 1B). The saturating model demonstrates a finite upper limit of chain extensibility, likely dictated by the inherent flexibility of the gelatin-thymol network, which correlates with the diminishing intermolecular cohesion: once thymol permeates the rigid cross-linked structure the polymer chains experience greater flexibility and thus greater ductility. Thymol continued to enhance flexibility at a slow saturation rate (c = 0.419), resulting in greater flexibility at higher thymol loadings and the films being more conformal for wound coverage. In summary, these results present an evident mechanical tradeoff: thymol increased flexibility (increase in EAB) at the detriment of mechanical integrity (decrease in TS and E). This corroborates reports from the literature referring to essential oil–biopolymer film [13]. For wound dressing applications, there needs to be a balance to take into account comparable strength for handling qualities, stiffness to maintain shape, and high elongation for the mobility of someone's tissue. Based on the models developed we define 2-4% thymol as the optimum window whereby TS is above 1.3 N/m^2 (sufficient to handle), and E is at or above 2.0 MPa (approximately no structure rigidity). E stays at or above 2.0 MPa (allowing little structural rigidity), and if EAB surpasses 150% (indicating excellent conformability). Once thymol is too high (greater than 4%), E drops sharply (<2.0 MPa) which may relate to the structural failure of the film during application, rather than further enhancements in flexibility.

The fitted models reproduce all experimental data points [12] with overall coefficient of variation of 8%, confirming their validity, therefore providing evidence for the model's credibility. Further, all functional forms are physically meaningful: the exponential decay represents the continual fracture of the gelatin network, while the saturating EAB curve model captures the limit where molecular chains cannot further contribute to an increase of EAB. These models provide a predictive means for modifying mechanical properties with respect to thymol concentration and facilitate the targeted design of gelatin-based nano wound dressings with desired mechanical properties. Collectively, thymol functions as a dual-use additive, imparting both antimicrobial and antioxidant properties [12] while altering mechanical properties. The quantifiable relationships established in this chapter will allow for multi-objective optimization of future bioactive wound dressings.

The Monte Carlo simulation framework was used to measure uncertainty in the prediction of the mechanical properties of thymol-loaded gelatin films and to assess the probability of exceeding unknown thresholds of each mechanical property in order to exhibit helpful function as a wound dressing material. Across factors of concentration, we can see clear differences for tensile strength (TS), Young's modulus (E), and elongation at break (EAB), which shows a tradeoff between integrity and flexibility of the films as desirable properties for WDs.

Fig. 2 shows the probability of exceeding a TS threshold of 1.5 N/m^2 as a function of the concentration of thymol in gelatin. Initially at 0% thymol, the model predicts nearly 100% probability (P > 0.99), which aligns with our experimental observation that cross-linked films with 0% thymol had the highest TS as measured (4.0 ± 0.4 N/m^2). This probability quickly declines after 1.2% thymol, becoming almost negligible by 1.6% Thymol. However, across all parameters measured we see that the addition of thymol disrupts the gelatin network disrupting intermolecular cohesion and crosslinking functionality. Therefore, if a mechanical property requires strong handling strength, we suggest that thymol loading should be limited to below 1.2% concentration.

The probability of E exceeding 2.0 MPa is illustrated in Fig. 3. Similar to TS, at low thymol concentrations (≤ 1.5%) the probability asserted high (>0.95) and decreased steadily with increased thymol loading. At 8% thymol, the probability leveled off to around 40%, suggesting that when most films will have E <2.0 MPa there are still significant number of films that may demonstrate sufficient rigidity in certain contexts. This means that some formulations for applications may still hold some structural rigidity with greater thymol loadings.

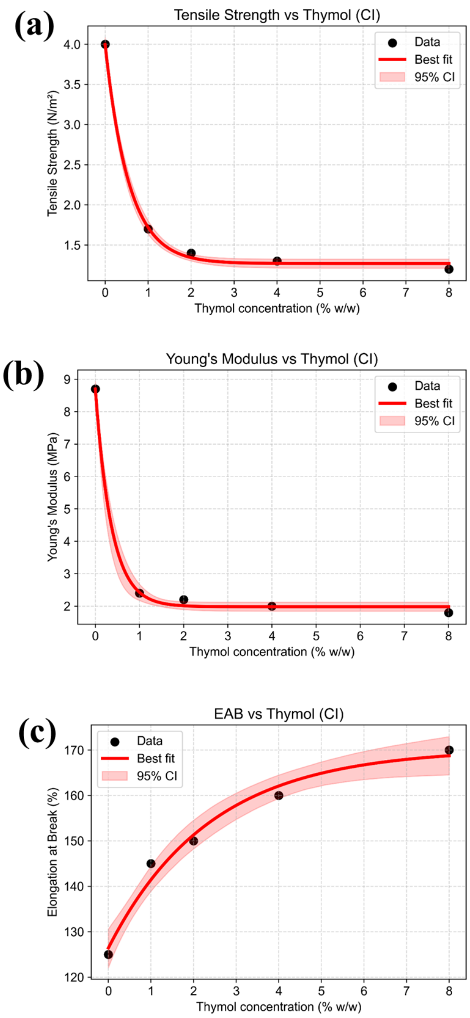

Fig. 1. (a) Tensile strength (TS), (b) Young's modulus (E), (c), Elongation at break (EAB) of cross-linked gelatin films as a function of thymol concentration (% w/w). Experimental data (mean ± SD, $n \geq 3$) [12] are shown as black circles. The red solid line represents the best-fit exponential decay model.

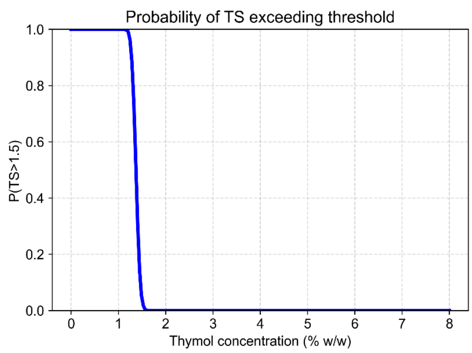

Fig. 2. Probability of tensile strength (TS) exceeding 1.5 N/m^2 as a function of thymol concentration (% w/w).

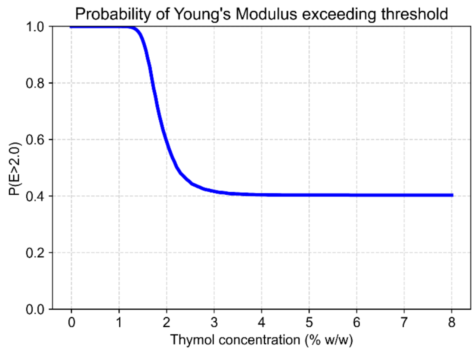

Fig. 3. Probability of Young's modulus (E) exceeding 2.0 MPa as a function of thymol concentration (% w/w).

Different from TS and E, Fig. 4 demonstrates that the probability of EAB exceeding 155% increases rapidly as thymol concentration increases, and is nearly certain ($P > 0.99$) by 2.5% thymol. Experimental data corroborate this result, with EAB increasing from 125% at 0% thymol to 170% at 8% thymol. Thymol increases chain mobility and plasticity which make the films more extensible, position them well for conforming to irregular wound surfaces.

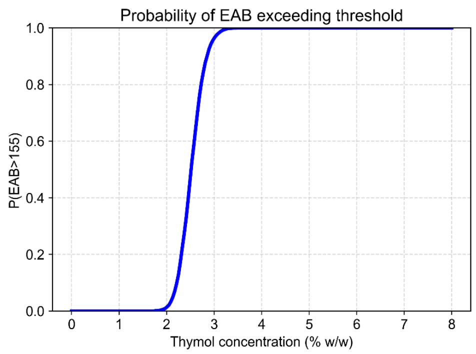

Fig. 4. Probability of elongation at break (EAB) exceeding 155% as a function of thymol concentration (% w/w).

The probabilistic curves generated in the above create a powerful method for rational design. For example, if a wound dressing seeks: High tensile strength ($TS > 1.5$ N/m^2) and moderate stiffness ($E > 2.0$ MPa), then a concentration of thymol would be optimal at ~1.0 – 1.2%. If high flexibility ($EAB > 155\%$) with adequate strength, a concentration of thymol can be maintained at ~2.0 – 3.0%, while if there is a premium on maximum antimicrobial properties (moving higher with increasing amounts of thymol), a concentration ≥4% is required, but with a lowered amount of both TS and E. This research shows that thymol was a strong modifier of mechanical behavior, allowing tunable film properties by controlling dosage. The Monte Carlo method reduces few experimental data to effective guidelines to keep in mind when designing. This work provides a consideration for the design of next-generation nano wound dressings, with a focus on the trade-off between bioactivity and mechanical performance.

4. Conclusion

This study presents modeling and probabilistic evaluation of the mechanical properties of thymol-loaded gelatin films, based on data reported exclusively by Kavoosi et al. [12]. Physically-informed empirical models scientifically collected and fitted, and which decay exponentially for both tensile strength and Young's modulus, and saturating, with respect to elongation at break, indicate how thymol affected the mechanical properties of the films. Monte Carlo simulation supported a clear mechanical trade-off: thymol improved gum films flexibility (increased EAB) at the same time reduced structural strength (decreased TS and E), by dislocating interaction between gelatin chains and partially inhibiting glutaraldehyde crosslinking. The probabilistic analysis of the films further quantified a range for which ~2-4% (w/w) thymol concentration provided the greatest likelihood of tensile strength > 1.2 N/m^2, elongation > 150%, and stiffness > 2.0 MPa. The 2-4% (w/w) range also provides for maximum bioactive properties of thymol and its reportedly antioxidant and antimicrobial properties, whilst also having requisite mechanical properties for functional wound dressing. Overall, the results developed through this modeling and probabilistic analysis form a risk-aware, quantitative design method to further gelatin-based nano wound dressings.

The probabilistic analysis identified an ideal range of thymol concentrations (2-4% w/w) that maximizes the chances of achieving mechanical target values for tensile strength (> 1.2 N/m^2), elongation (>150%) and stiffness (> 2.0 MPa) concurrently. This concentration range is a balance of the bioactivity of thymol and its demonstrated antioxidant and antimicrobial activity with the mechanical performance needed to take the form of a functional wound dressing.

These findings provide a quantitative, risk-informed design framework for the formulation of gelatin-based nano-wound dressings. By designing the mechanical performance with a predictable dose of thyme essential oil, it expresses the basis for rational engineering of bioactive films with therapeutic effect and mechanical robustness. Future work should use these mechanical models in tandem with in vivo performance models to ascertain their clinical translatability.

Author contributions

Nadia Banitorfi Hoveizavi: Investigation, Conceptualization, Writing – original draft, Writing – review & editing; **Mastafa H. Al-Musawi:** Conceptualization, Writing – original draft, Writing – review & editing.

Funding

No funding was received for this study.

Conflict of interest

The authors declare no conflict of interest.

Data availability

No data is available.

REFERENCES

[1] M. Gómez-Guillén, B. Giménez, M.a. López-Caballero, M. Montero, Functional and bioactive properties of collagen and gelatin from alternative sources: A review, Food hydrocolloids 25(8) (2011) 1813-1827.
[2] J.S. Boateng, K.H. Matthews, H.N. Stevens, G.M. Eccleston, Wound healing dressings and drug delivery systems: a review, Journal of pharmaceutical sciences 97(8) (2008) 2892-2923.
[3] K. Vimala, Y.M. Mohan, K.S. Sivudu, K. Varaprasad, S. Ravindra, N.N. Reddy, Y. Padma, B. Sreedhar, K. MohanaRaju, Fabrication of porous chitosan films impregnated with silver nanoparticles: a facile approach for superior antibacterial application, Colloids and Surfaces B: Biointerfaces 76(1) (2010) 248-258.

[4] L.-H. Li, J.-C. Deng, H.-R. Deng, Z.-L. Liu, X.-L. Li, Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films, Chemical Engineering Journal 160(1) (2010) 378-382.
[5] K. Can Baser, Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils, Current pharmaceutical design 14(29) (2008) 3106-3119.
[6] C.-C. Huang, H.-F. Wang, C.-H. Chen, Y.-J. Chen, K.-H. Yih, A study of four antioxidant activities and major chemical component analyses of twenty-five commonly used essential oils, Journal of Cosmetic Science 62(4) (2011) 393-404.
[7] R. García-García, A. López-Malo, E. Palou, Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua, Journal of food science 76(2) (2011) M95-M100.
[8] J. Xu, F. Zhou, B.P. Ji, R.S. Pei, N. Xu, The antibacterial mechanism of carvacrol and thymol against Escherichia coli, Letters in applied microbiology 47(3) (2008) 174-179.
[9] A. Rao, Y. Zhang, S. Muend, R. Rao, Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway, Antimicrobial agents and chemotherapy 54(12) (2010) 5062-5069.
[10] A. Ahmad, A. Khan, F. Akhtar, S. Yousuf, I. Xess, L. Khan, N. Manzoor, Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida, European journal of clinical microbiology & infectious diseases 30(1) (2011) 41-50.
[11] D. Tasdemir, M. Kaiser, F. Demirci, K. Baser, Essential oil of Turkish Origanum onites L. and its main components, carvacrol and thymol show potent antiprotozoal activity without cytotoxicity, Planta Medica 72(11) (2006) P_077.
[12] G. Kavoosi, S.M.M. Dadfar, A.M. Purfard, Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing, Journal of Food Science 78(2) (2013) E244-E250.
[13] P. Tongnuanchan, S. Benjakul, T. Prodpran, Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils, Food chemistry 134(3) (2012) 1571-1579.