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Novel Tri-biopolymer Thin Films with Superior Antimicrobial Efficacy

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Novel Tri-biopolymer Thin Film with Superior Antimicrobial Efficacy

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Physicochemical Properties

Abstract

This work reports a novel synthesis, characterization, and performance of antimicrobial polymeric thin films utilizing unreported ternary blend of Chitosan, PLA, and PCL. For binary systems, the selection of matching polymers has improved the material's structures and properties; however, the transition to using a ternary system is not well-established yet. The ability to homogeneously blend three biopolymers would expand the potential to prepare new materials with tailored properties. Herein, unary, binary, and ternary films were produced by physical blending using the casting and solvent evaporation method. The films were then characterized using different techniques for comparing the properties of three mixing ratios of ternary films with the unary and binary recipes of the selected polymers. The ternary films showed homogenous chemical structure, thermal stability up to 400 °C, enhanced solubility, with adequate mechanical and structural integrity. These results exhibited the potential for enhancement as we transitioned from unary to ternary blends, resulting in a notable improvement in antibacterial behavior. Specifically, the antimicrobial effects of these blends against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* microbes have been reported.

1 Introduction

The process of synthesizing and producing functional and robust thin films using bio-based polymers with specific properties and for targeted applications is strenuous and requires uncertain efforts and massive financial requirements. These cues motivated the researchers to focus on blending of already existed biopolymers with known and well-defined properties to prepare materials with tailored and highly controlled features. The polymers selection is mainly related to both the pre-designed properties and the intended application. Traditionally, fossil-based materials are widely used for general thin films applications. These materials represent a serious environmental threat, and their prices are affected by the cost of oil¹; therefore, intensive research has been performed on investigating the use of biodegradable polymers to develop renewable, eco-friendly, biodegradable, and cost-effective film packaging technologies². Recently, research

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areas, including biomaterial synthesis and processing, are increasingly interested in using bio-based and biocompatible synthetic polymers. These polymers proved desirable properties for synthesizing biodegradable, biocompatible, and bioresorbable materials for several interesting medical applications.^{3,4}

Among a wide board of polymers, Chitosan (CS), poly(lactic acid) PLA ($C_3H_4O_2$)_n, and poly(ϵ -caprolactone) PCL ($C_6H_{10}O_2$)_n are widely used for different applications because of their robust and distinguished properties. Chitosan is a polysaccharide obtained from the deacetylation of chitin, which exists naturally in shellfish skeletons, including crab, lobster, and shrimp. Chitosan is an attractive candidate for biomaterial development applications because of its biodegradability, biocompatibility, promoting cell adhesion in addition to antibacterial activity.^{5,6} PLA is a biocompatible and biodegradable polymer that is easily processed and has high stiffness and mechanical strength⁷. Likewise, PCL is biodegradable, biocompatible, and exhibits tunable mechanical properties; however, these polymers individually have significant drawbacks, which limit their applicability as pure materials. For instance, CS is brittle and water sensitive, and PCL has a slow degradation rate, poor cell adhesion due its high hydrophobicity, and poor antibacterial and mechanical properties.⁸⁻¹⁰ PLA also has low elasticity, weak cell adhesion, biological inertness, slow degradation, and degrades into acidic by-products.¹¹

Numerous research efforts have focused on enhancing PCL, PLA, and Chitosan properties to resolve the shortcomings for different applications.¹²⁻¹⁴ For instance, the manufacturing of Chitosan films by thermoforming or solvent-free techniques is not achievable as its melting temperature is more than the degradation temperature. However, reliable Chitosan films were fabricated by Sun et al. for biodegradable electronics applications through roll-forming of Chitosan blends.¹⁵ This blend employed the ionic liquid (1-Butyl-3-methylimidazolium chloride) plasticizing effect to introduce better processability for the neat Chitosan. Although the enhanced capability of manufacturing Chitosan film at nominal conditions, the produced films required additional step of removing the ionic liquid from the Chitosan structure which may affect the film's mechanical integrity.

Interestingly, polymer blending is a promising methodology for overcoming these challenges and obtaining reliable polymeric film properties.^{9,16-18} Basically, the blending technique depends on mixing two or more polymers to produce a new blended material with new and enhanced physicochemical properties.^{19,20} For example, the PLA and PCL blending compatibility was significantly improved with enhanced mechanical properties by the uniform scattering of PLA nanofibers within the PCL matrix as proposed by Kakroodi et al.²¹ However, there is still a concern regarding the PLA fiber synthesis as the droplet dispersion quality determines the dispersion condition of the nanofibers affecting the process reproducibility. Also, Semnani et al. investigated scaffold fabrication utilizing the electrospinning of Chitosan and PCL nanofibers for liver tissue engineering applications. They designed a novel setup for collecting the nanofibers with improved orientation and controlled pore size suitable for the infiltration of the cultured cells.²² The electrospinning of the PCL nanofibers with Chitosan nanoparticles is also employed by Jung et al. to synthesis a nanocomposite for drug delivery and wound dressing applications.²³ Nevertheless, most of these fabrication techniques are not fully controllable and hard to be precisely reproduced. Furthermore, Yoshida et al. utilized chitosan to improve the bioactivity of the PCL scaffold for promoted culturing of mesenchymal stem cells (hBMSCs) by employing the 3D melt-electrowritten technique.²⁴ The authors obtained a significant increase in hBMSC proliferation rate (~140%) with a 1 wt% chitosan-PCL scaffold compared to a neat PCL scaffold. Carette et al. immobilized chitosan on the surface of PLA using a microwave atmospheric plasma and dip coating.²⁵ The chitosan provided antimicrobial activity for the PLA surface, allowing the tailored PLA to offer many attractive properties for industry and tissue engineering applications. Demchenko et al. synthesized a PLA-chitosan with an Ag⁺ ion biocompatible nanocomposite with good antibacterial and antiviral activities.²⁶ This process was optimized by manipulating the

concentration of the silver ions. However, many efforts also studied the feasibility of modifying a polymer structure using organic, inorganic, biological constituents, and other materials.^{27–29} There have been no prior reports on the successful blending of the three most common biodegradable polymers, specifically Chitosan, PLA, and PCL, at three different ratios to the best of the author's knowledge.

In this work, we report the successful preparation of unary, binary, and ternary films of Chitosan, PLA, and PCL with homogeneous and enhanced physicochemical properties. We tested different recipes of Chitosan, PLA, and PCL aiming to obtain thin films with significantly improved physiochemical and antibacterial properties. The casting and solution evaporation method was utilized to prepare the films using different ratios to evaluated the blending matching at different levels. To the best of the author's knowledge, no work has been reported on uniformly blended films of these three polymers at different mixing ratios. The feasible preparation process allowed us to optimize and tune the chemical composition, mechanical, optical, and thermal properties of the produced films. The ternary films exhibited an excellent antibacterial efficacy against negative and positive-gram bacteria. This study marks the beginning of a research path towards exploring more useful blends for biomaterials processing and development applications.

2 Methods and Experiments

2.1 Materials

Shrimp shell-derived chitosan with a deacetylation degree of $\geq 75\%$ was obtained from Sigma-Aldrich, Iceland. Polycaprolactone PCL pellets (average $M_n=80,000$) were obtained from Sigma-Aldrich, United Kingdom. Polylactic acid PLA 8302D pellets were supplied by NatureWorks company, USA. Acetic Acid Glacial (CH_3COOH) and chloroform (CHCl_3) were purchased from Matheson Coleman & Bell, USA, and MilliporeSigma, Germany, respectively.

2.2 Blending Methods

The three parent solutions of Chitosan, PLA, and PCL were prepared separately. Chitosan powder was dissolved in 0.5 M acetic acid at 1.0% (w/v) under vigorous stirring at 50 °C for six hours. The solution was allowed to cool down for 24 hours at room temperature, then vacuum filter system was used to remove the precipitated molecules followed by air bubbles elimination by degassing. The PCL and PLA were prepared at the same weight to volume ratio of 5% (w/v) by dissolving the polymers into chloroform under stirring at room temperature for six hours. After that, pure, binary, and ternary blended films were then produced using the film casting method as follows:

- i) A Chitosan pure film was produced by solvent evaporation of 10 ml of the prepared chitosan solution in a glass petri dish (6 cm diameter) at 60 °C for 24 hours. The PCL and PLA films were also prepared by diluting 2.0 ml of their solutions into 8.0 ml of chloroform and then evaporating the solvent for 24 hours at 30 °C. The dried films were then peeled off and kept in a plastic petri dish.
- ii) The CS+PCL or CS+PLA blended films were prepared by adding 1.0 ml of PCL or PLA solution to 10 ml of chitosan solution dropwise with stirring at 30 °C for two hours. The solution was stirred at 50 °C for 30 minutes to evaporate the remaining chloroform before degassing the solution in a vacuum pressure for 20 minutes. This solution was poured into a glass Petri dish and dried at 30 °C for 24 hours before the film was peeled off. The PCL+PLA blend film was made by mixing 0.75 ml of the PCL and PLA solutions in 10 ml of chloroform and stirred for six hours at room temperature. The solution was then degassed by vacuum, cast in a petri dish, and dried overnight to produce a homogeneous film.
- iii) The ternary blended films were prepared by adding a PCL solution dropwise to 10 ml of CS solution at 30 °C under stirring for one hour before adding PLA-CS in the same manner. The mixed solution

was degassed, cast, and dried at 30 °C for 24 hours. These steps were followed to prepare three films with different PCL and PLA weight ratios. Table 1 summarizes the prepared films and their polymeric constituent percentages.

Table 1: Summary of biopolymeric content in the produced blend films.

Film	CS %	PLA %	PCL %
CS	100	0	0
PLA	0	100	0
PCL	0	0	100
CS+PLA	67	33	0
CS+PCL	67	0	33
PLA+PCL	0	50	50
CS+PLA+PCL	67	16.5	16.5
CS+PLA (23%)+PCL(10%)	67	23	10
CS+PLA(10%)+PCL(23%)	67	10	23

2.3 Characterization

2.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of the Chitosan, PCL, and PLA neat, binary, and ternary blend films were investigated using a Bruker Vertex 70 spectrometer (Bruker Optic GmbH, Ettlingen, Germany). The spectra of the studied samples were obtained at room temperature in attenuated total reflectance (ATR) mode with a diamond crystal within a range of wavenumbers between 600-3800 cm⁻¹, with 32 scans for each sample and resolutions of 4 cm⁻¹. All spectra were further analyzed using OPUS 7.5 software (Bruker Optic GmbH, Ettlingen, Germany).

2.3.2 Thermogravimetric (TGA) and Differential Thermogravimetric (DTG) Analysis

The prepared films' thermal stability was tested using thermogravimetric measurements (TGA) and differential thermogravimetric DTG analysis. TGA data were acquired using a Thermal Analysis SDT Q600 analyzer (TA Instruments, Champaign, IL, USA). Approximately 5 mg of the film was heated from room temperature to 840 °C under a nitrogen gas flow of 100 cm³/min with a heating rate of 10 °C·min⁻¹. The thermograms were analyzed using TA universal analysis software. DTG curves were recorded by differentiating TGA values with respect to time following the central difference method as follows:

$$DTG = \frac{W_{t+\Delta t} - W_{t-\Delta t}}{2\Delta t}$$

Where $W_{t+\Delta t}$ and $W_{t-\Delta t}$ are the residual weights of the specimens at time points $t+\Delta t$, and $t-\Delta t$, respectively. Δt is the time duration between two residual weights.

2.3.3 Film Thickness

The film thicknesses were measured using a portable micrometer (Starrett 436. 1RL-1, Massachusetts, USA) with an accuracy of 0.0001 inches. Five measurements were taken at different film positions to calculate average thickness, decreasing random error. The averaged thickness values were further used for opacity calculations.

2.3.4 UV-Visible Spectroscopy

A Thermo Scientific Evolution 600 UV-Vis Spectrophotometer was employed to analyze the transmittance and absorbance ratios of the pure, binary, and ternary blended films at wavelengths ranging between 250–850 nm. This test was performed by cutting film samples to fit the spectrophotometer cuvette and analyzed using transmittance mode. The transparency was calculated using the following equation:

$$opacity = \frac{\%T_{600}}{d}$$

T_{600} is the transmittance percentage at a wavelength of 600, and d is the sample's thickness in millimeters.

2.3.5 Water Solubility

Water solubility is described as the percentage of dry matter dissolved after soaking the sample in distilled water for 24 hours. The resultant water solubility was investigated by modifying the method described by Fabio et al.³⁰. The films were cut into squares with an area of 2 cm² and dried at 80 °C for 48 hours. The samples were then weighed and soaked in 10 ml of distilled water in closed beakers for 24 hours. The films were filtered out, dried at 80 °C for 48 hours, and their weights were recorded. The water solubility was calculated using the following equation:

$$Water\ solubility\ \% = \frac{M_{initial} - M_{final}}{M_{initial}} \times 100$$

Where $M_{initial}$ and M_{final} are the dried film masses before and after the dissolving process, respectively.

2.3.6 Mechanical Properties.

The nanoindentation test yields a valuable view of a material's mechanical properties in a fast and feasible manner. The tests were performed using a Hysitron TI 700 UBI (Minneapolis, MN) with a Berkovich diamond indentation tip to obtain the prepared films' hardness and Young's modulus. Neat and blend films with diameters of 4.7 mm and approximate thicknesses of 50 µm were attached to aluminum stubs using super glue. The trapezoidal loading function was selected to conduct the test, loading the sample from 0 to 500 µN linearly during 10 seconds, holding the load for 10 seconds, and then unloading for another 10 seconds. The test was repeated at 16 points for each sample, and a space interval of 400 µm between every two points was used to avoid any possible interactions. The OriginPro8 software was also used to analyze the obtained load-displacement curves and calculate sample hardness and Young's modulus.

2.3.7 Antibacterial Test

Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) were used to evaluate the antimicrobial efficacy of the six blend films using the agar diffusion test. Sterile loops were used to extract individual microbes from their pure cultures. Typical Soy Agar was carefully inoculated under a fume hood with either *E. coli* or *S. aureus* at room temperature. The microbes were spread further by forming a lawn of microbial growth. Neat, binary, and ternary blend films were cut into 4.7 mm diameter discs using a standard paper puncher. The samples were sterilized using ethanol, placed on the agar, then incubated for 24 hours at 37 °C. The antimicrobial effect of each film was recorded by measuring the average diameter of the efficacy zones caused by the films on the cultured agar plates.

2.3.8 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS).

The surface and cross-sectional views of the ternary film (67% CS, 16.5% PLA and 16.5% PCL) were subject to SEM (Hitachi SU8010) with EDS to investigate both the microstructure and the elemental composition homogeneity of the blended polymers, respectively. To acquire an improved cross-sectional view, the sample was cut from the film and immersed in liquid nitrogen to freeze and then snap it from the middle to provide an even edge. Then, the sample was fixed on the double face for carbon sputter coating preparing it for SEM and further EDS analysis.

3 Results and Discussion

3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

ATR- FTIR spectroscopy was used to identify the functional groups present, the intermolecular-intramolecular interactions, and to verify complexation taking place in the Chitosan, PLA, PCL, pure, and blend films. Figure 1 depicts the FTIR spectra of the neat, binary, and tertiary blend films made of PLA, PCL, and Chitosan. The characteristic spectrum of the chitosan film demonstrates absorption bands around 1638 (C=O stretching), 1530 (N-H bending), and 1371 cm^{-1} (stretching of the C-N of N-acetyl residues), which agrees with the literature. The broad absorbance at 3370 cm^{-1} is attributed to the O-H and N-H bond's stretching vibration, while sharp peaks at 1150 and 1008 cm^{-1} represent chitosan's saccharide structure^{31,32}. The PCL film spectrum exhibits a sharp peak at approximately 1720 cm^{-1} , representing Carbonyl stretching, and other peaks at approximately 2863 cm^{-1} and 2941 cm^{-1} identify the symmetric and asymmetric CH_2 stretching within the PCL polymeric chain. The peaks at 1157 and 1291 cm^{-1} correspond to C-O and C-C stretching in the PCL crystalline and amorphous phases, respectively³³. The neat PLA's FTIR spectrum demonstrates a strong absorbance at 1743 cm^{-1} due to the carbonyl group's C=O stretching. The amorphous and crystalline PLA phases are also depicted as absorbance bands at approximately 871 cm^{-1} and 738 cm^{-1} , and the band at approximately 2950 cm^{-1} represents the symmetric and asymmetric C-H stretching in CH_3 . Bands at 1064 and 1184 cm^{-1} are related to the C-O bond stretching vibration³⁴. The FTIR results of the blend films appeared as an overlapping of the blended polymers' spectra with noticeable differences in band positions, sharpness, and intensities. The characteristic peaks of neat CS at 1638 and 1530 cm^{-1} are shifted to 1652 and 1538 cm^{-1} in the CS+PLA film, respectively. The PLA's characteristics peaks at 1743 and 738 cm^{-1} appeared sharper at higher wavenumbers of 1747 cm^{-1} and 749 cm^{-1} , respectively. Blending PCL with CS reduced the absorbance intensity of PCL's peaks at 2863 and 2941 (CH_2 stretching) and shifted their positions to 2858 and 2933 cm^{-1} , respectively. Further alterations occurred in the CS-PLA-PCL film, where the sharp peak at the 1150 cm^{-1} carboxyl stretch in CS disappeared, and the carbonyl stretching peaks in PCL (1720 cm^{-1}) and PLA (1743 cm^{-1}) overlapped, forming one broader peak at 1722 cm^{-1} . The N-H and C-H stretching peaks at 3370 cm^{-1} became broader and more intense. These pronounced changes are a direct indication of complexation taking place between the three polymers, and therefore can be attributed to the successful blending of the three polymers. Based CS-PLA-PCL spectra, it is evident that all the characteristic peaks of CS and PLA/PCL were observed at 3,370, 2,936, 1,722, and 1,559 cm^{-1} . This indicates successful and homogenous blending of the three-polymer using the simple and straightforward physical blending.

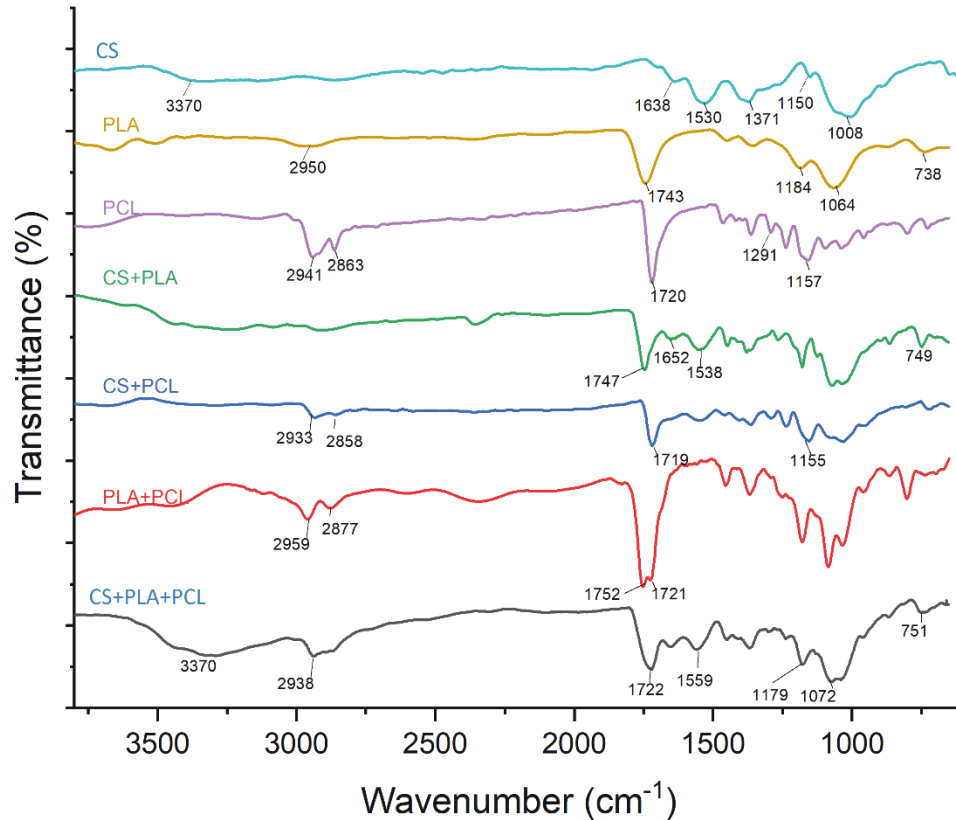
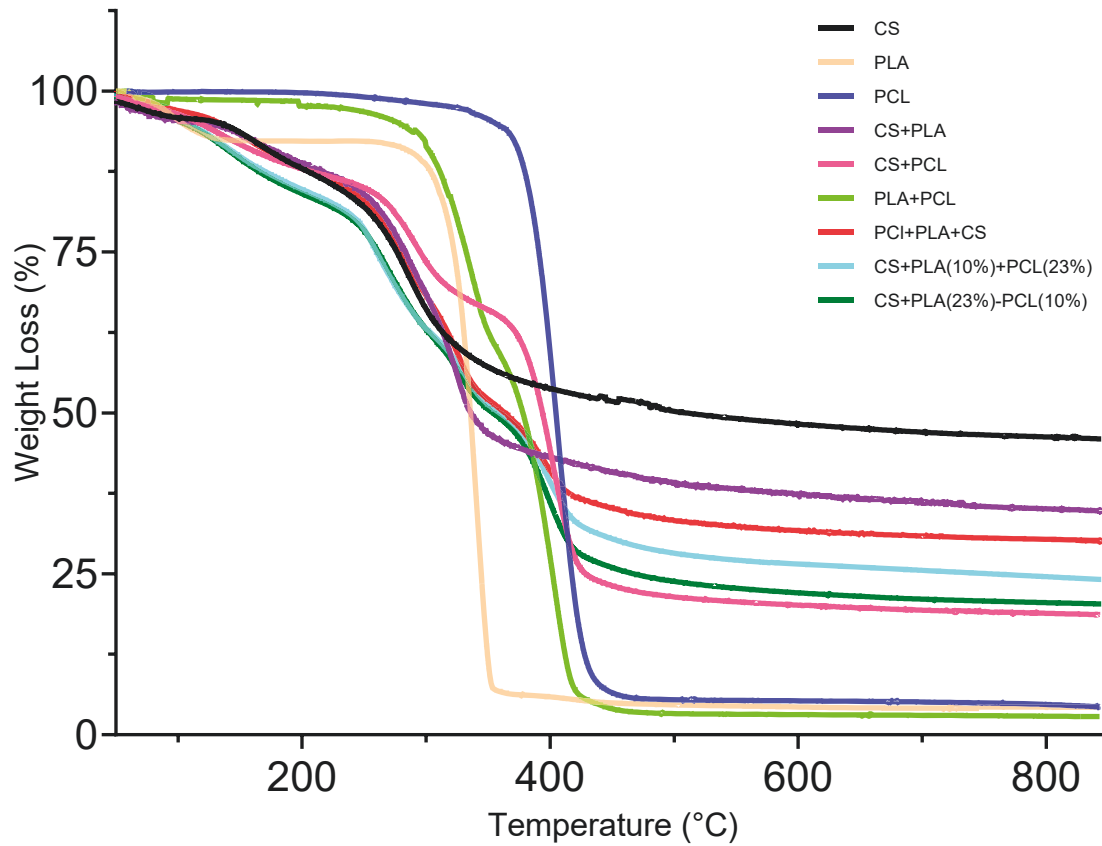


Figure 1. FTIR spectra of the neat and blend Chitosan, PLA, and PCL films.

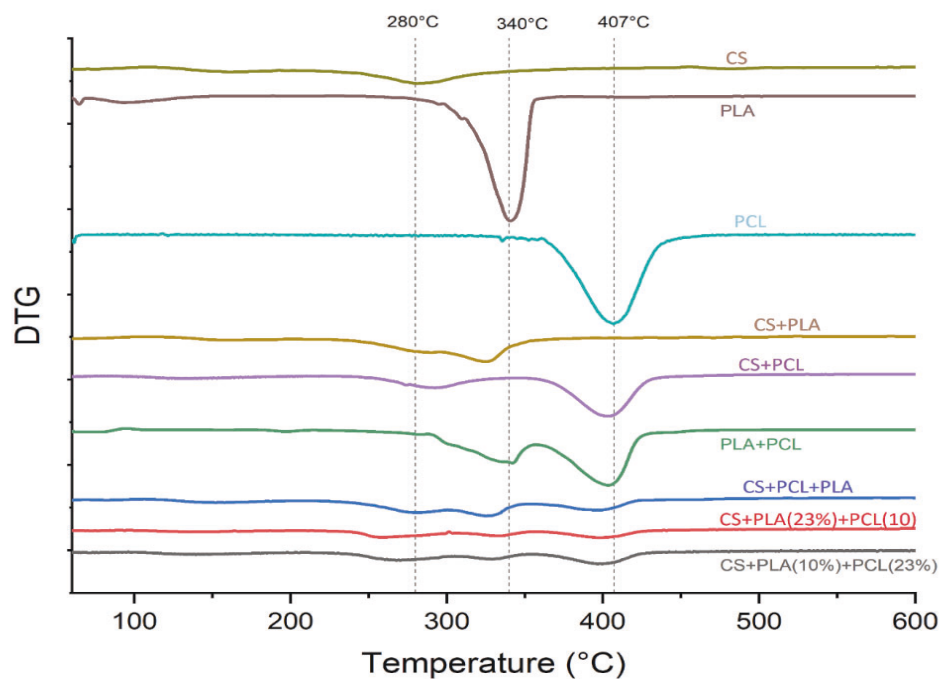
3.2 Thermogravimetric (TGA) and Differential Thermogravimetric (DTG) Analysis

Investigating the blend films' thermal stability is crucial since they are subjected to heat exposure while processed or consumed. Also, assessing the thermal behavior of the blend is useful for assessing how thermal transition of the prepared films is affected by the successive addition of the blend components. Thermogravimetric analysis (TGA) was used to determine the produced blend films' stability upon heating within a temperature range from 25 °C to 840 °C, with an incremental step of 10 °C per minute. TGA and DTG thermograms were obtained to determine the film's weight loss and degradation rate under the influence of temperature (Figure 2). There are two weight loss stages for the pure chitosan film. The smaller step is between 50 and 200 °C, corresponding to the evaporation of the acetic acid residuals and water. The second degradation step appears at 280 °C due to chemical degradation³⁵. TGA and DTG curves indicate that PLA and PCL pure films degrade in a similar single-step manner: at maximum degradation rates around 340 °C and 410°C, respectively.^{36,37} The TGA and DTG curves for the binary and ternary blend film analysis demonstrated interfering thermograms composed of the graphs of the ingredient polymers with a slight shift in the degradation cut-off to lower temperature values. DTG curves of the binary blends demonstrated that blending PLA within chitosan decreased PLA's degradation temperature by 16 °C to 324 °C, while the degradation temperature of PCL in the CS+PCL films decreased by 8 °C to 402°C, indicating that PLA compatibility is higher than PCL in the Chitosan film. This behavior indicates enhanced blending between Chitosan and PLA compared to CS+PCL binary film. The CS+PLA+PCL film degraded maximally at three stages: 280, 325, and 395 °C; however, the maximum weight loss percentage, depicted as the plateau regions in the thermographs, differs depending on the type and ratio of the composition

polymers in the films. These results verify that the blending process for the partially miscible PCL, PLA, and chitosan biobased polymer blend is successful. Additionally, the improved thermal stability beyond 400 °C of the blend compared to neat PCL and PLA can be attributed to Chitosan content and the formed intermolecular interaction among the blend components as detailed in FTIR analysis.



(a)



(b)

Figure 2. (a) Thermogravimetric (TGA) and (b) Differential Thermogravimetric (DTG) analysis of the films.

3.3 Thickness and Water Solubility

One of the significant issues with Chitosan and other polysaccharide films is high-water sensitivity. Investigating film solubility in water is one of the most direct and simplest methods to provide insight into the films' hydrophilicity or hydrophobicity and their behavior in aqueous environments.^{38,39} The water solubility test acts as bench-stick for other water based solubility analysis as a greater degree of hydrolysis is reported for polymers in the presence of mono/divalent ions.⁴⁰ Table 2 summarizes the water solubility of the films produced in this work. The pure chitosan film demonstrated a solubility of approximately 30% due to its functional groups' high capacity to bind with water molecules. At high salinity, the solubility of pure chitosan-based polysaccharides has been reported to decrease due to lack hydrolysis process initiation to maintain the mechanical shearing effect with protected backbone. Same trend can be observed with hydrophobic PLA and PCL addition.^{41,42} The solubility was decreased to approximately 20% by blending Chitosan with the PLA and PCL hydrophobic polymers. However, for the tri-blend solubility, equal amount of PCL and PLA with 67% of chitosan shows the lowest water solubility or higher water resistivity. Between PCL and PLA, PLA shows higher potential for decreasing tri-blend water solubility. The solubility study shows the chitosan-based film's water resistivity and their durability can be modified for specific packing applications. Table 2 lists the average values of the film's thickness measured by the portable micrometer.

Table 2. Summary of the produced film's thickness, Abs₆₀₀, opacity, and water solubility.

Film	Thickness (μm)	Abs600	Opacity	Water Solubility %
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CS	57.4	0.05	0.87	30.1
PLA	56.39	0.07	1.24	Not soluble
PCL	51.39	0.36	7.01	Not soluble
CS +PLA	54.27	0.87	16.03	21.8
CS +PCL	55.88	0.59	10.56	24.5
PLA+PCL	48.76	1.57	32.2	Not soluble
CS +PLA+PCL	51.8	1.02	19.69	19.7
CS +PLA(23%)+PCL(10%)	50.82	1.1	21.65	21.3
CS +PLA(10%)+PCL(23%)	50.89	0.96	18.86	22.6

3.4 Optical Transparency

The produced films' optical properties, depicted in Figure 3, are related to the end user's product desirability due to their direct effect on the packaged products' appearance and usefulness. Figure 4 illustrates the transmittance percentage of the neat chitosan, PCL, and PLA films and their binary and ternary blends at wavelengths ranging from 250–850 nm. The results indicate that CS and PLA neat films are the most transparent of the studied films. The Chitosan film's transmittance is over 80% at wavelengths higher than 400 nm, whereas the PLA film's transmittance was between 70 and 90% at the same range. The PCL neat film spectrum demonstrates lower transparency than PLA and Chitosan: the PCL transmittance was between 15 and 44% in the studied region. The PCL+PLA film exhibited the highest opacity in the visible range, with a percent transmittance lower than 10%. The films' transmittance percentage decreases significantly when blending more than two polymers, which reflects the low miscibility of the used polymers at the molecular level. The absorbance at the visible range of 600 nm and the films' calculated opacity are summarized in Table 2. According to the calculated results, ternary films are excellent candidates for food packaging and other products that require protection from visible light spectrum. These films allowed about 10% or less of light in the 400 to 700 nm range to pass through and even less transmittance at UV range as shown in UV-Vis spectra in figure 4 which expands potentials to be used as UV protective films.

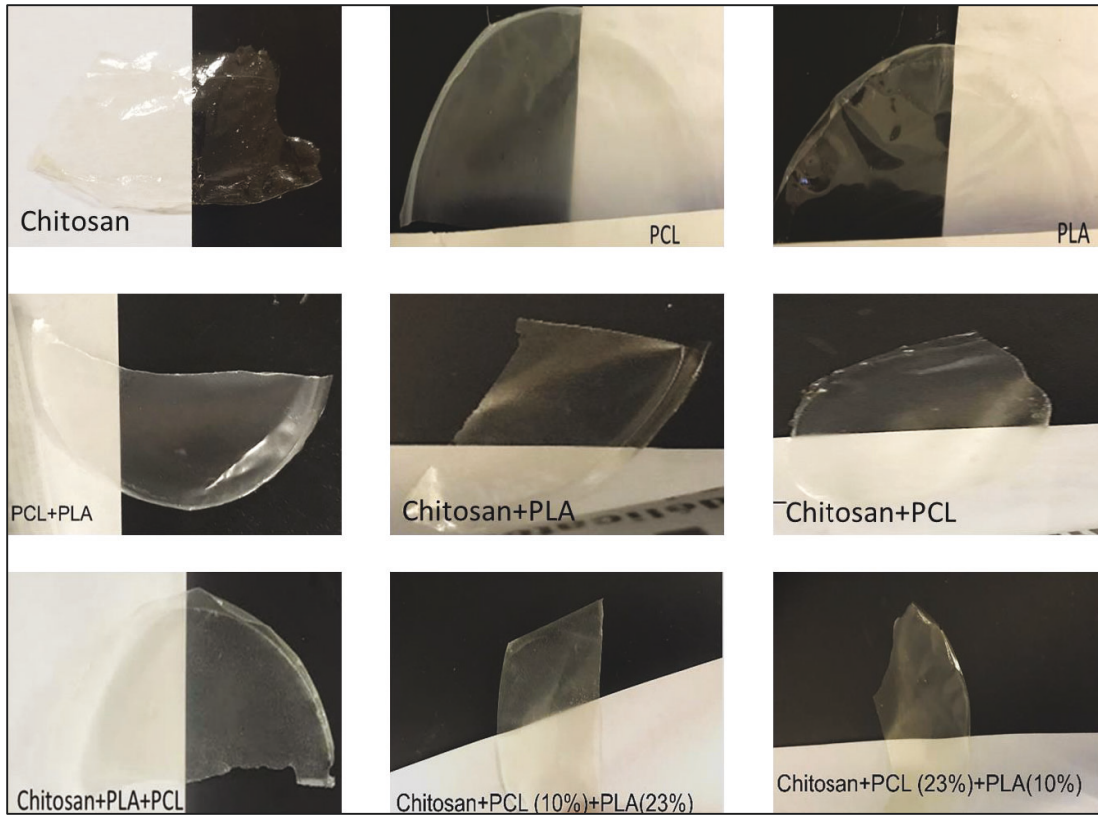


Figure 3. Pictures of the prepared films for the optical transparency test.

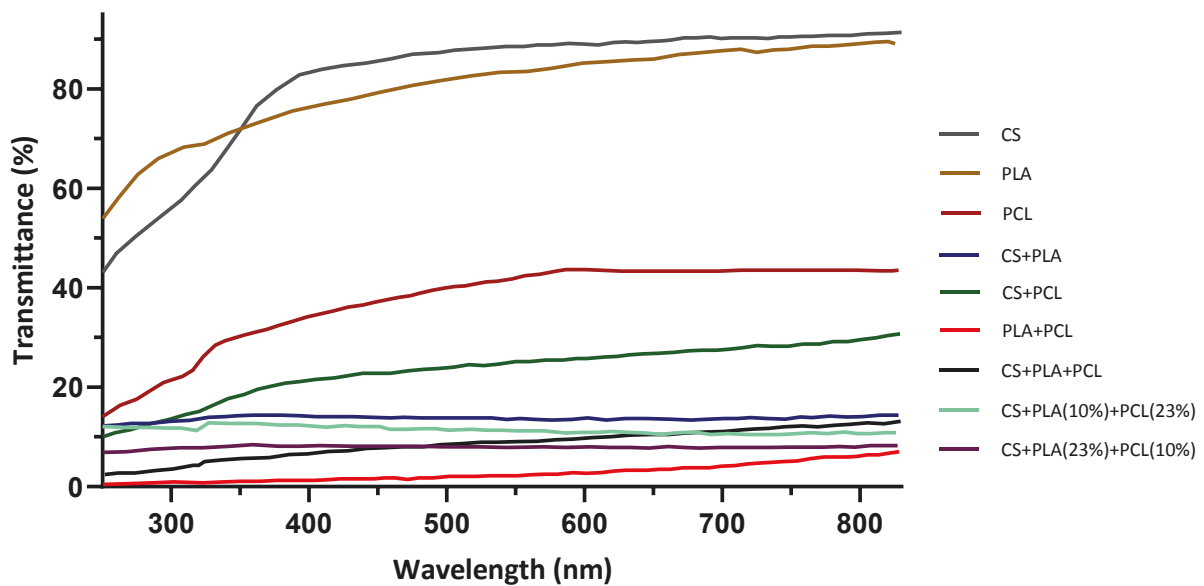
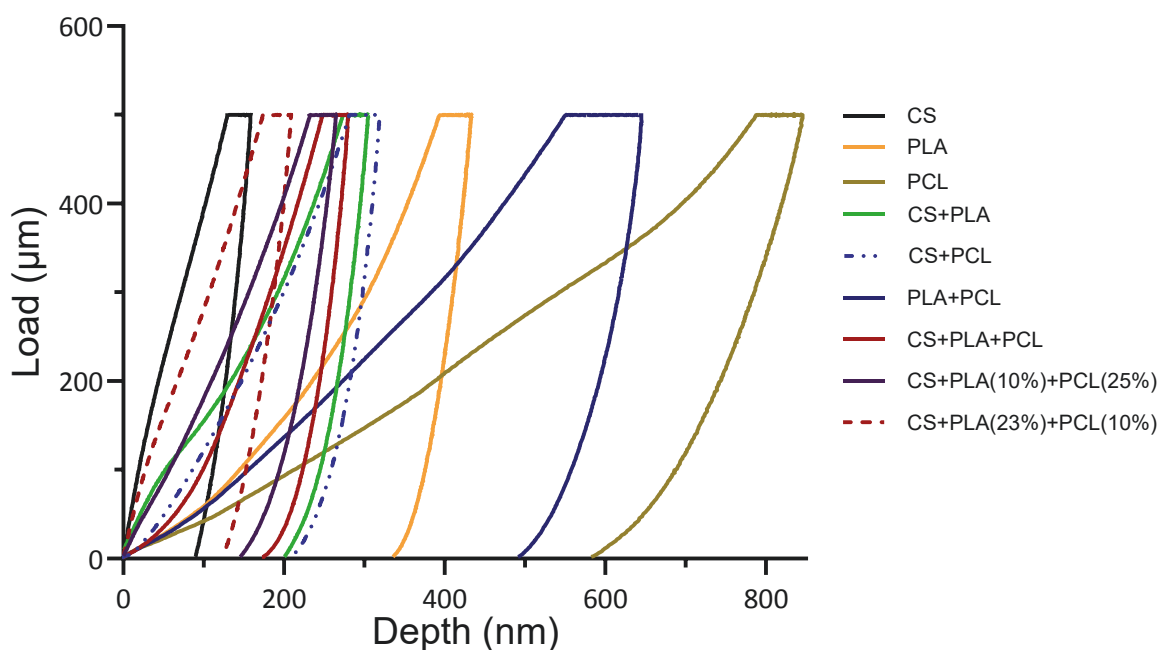
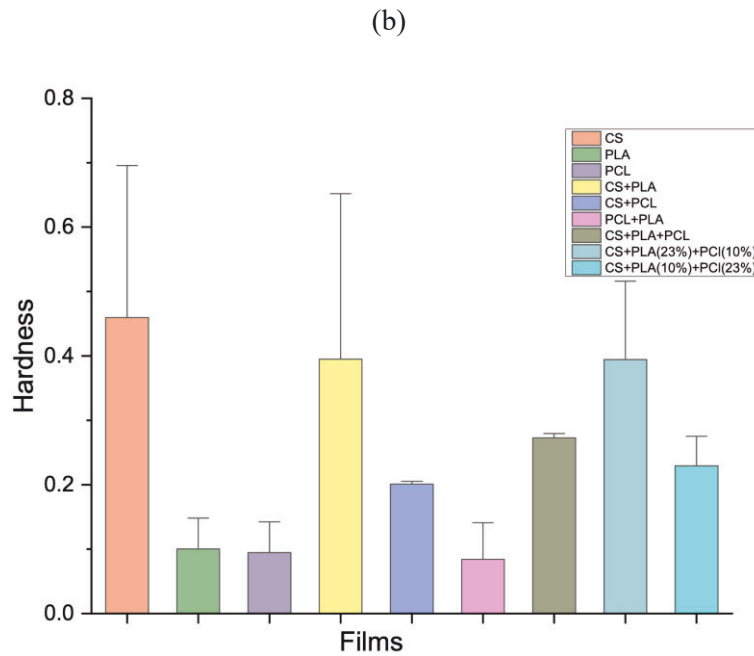
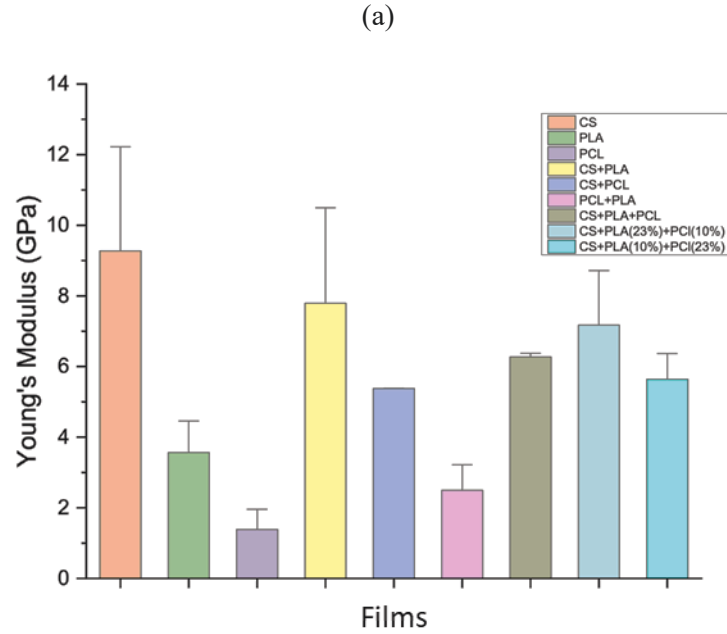


Figure 4. UV-Vis spectra of the produced films at wavelengths ranging between 250 and 850 nm.

3.5 Hardness and Young's Modulus

Figure 5 demonstrates the prepared films' mechanical properties, including load-displacement curves with mean Young's modulus and hardness values. The indentation areas were chosen carefully using in-situ SPM imaging from the nanoindentation instrument. The measurements were performed at the load control mode to eliminate the effects of surface roughness on the obtained results. The test was conducted 16 times on each sample, and the *Originpro8* software was used to analyze the obtained load-displacement curves and calculate Young's moduli. The outlier points were excluded, and the mean values ($p > 0.05$) for the obtained Young's modulus and hardness are presented in Figures 5b and c, respectively. The neat chitosan film exhibited the lowest indentation depth (< 200 nm) at the maximum load, while the PCL film achieved the highest value of ~ 846 nm. The maximum indentation displacement on the PLA film was approximately 430 nm. Young's modulus and the hardness of the pure chitosan films were the highest among the produced films ($E \sim 9.28$ GPa, and Hardness ~ 0.46), which can be attributed to the film's inherent brittleness, high molecular weight, low water content due to the relatively high temperature, and time duration used through the drying step. The Young's modulus and hardness values were approximately 3.72 GPa and 0.10 for PLA and 1.39 GPa and 0.095 for PCL, respectively. The blend's mechanical behavior varies depending on the type and concentration of the contained polymers. For example, we recorded Young's Modulus and hardness values of 7.79 GPa and 0.39, respectively, by blending CS with PLA, while these values were approximately 2.49 GPa and 0.084 for the PLA+PCL blends. The mechanical properties differed among the ternary blends. CS+PLA+PCL yielded a modulus value of 6.27 GPa and a hardness of 0.27; however, the modulus increased to 7.17 GPa, and the hardness increased to 0.39 when the PLA content was increased to 23%. Decreasing the PLA content to 10% produced a film with Young's modulus and hardness values of 5.64 GPa and 0.23, respectively. The consistency of the obtained mechanical properties confirms the successful blending of the three attractive polymers, allowing us to tailor their mechanical properties for a wide range of applications. These findings can aid in mimicking the mechanical properties of different human tissue types to produce effective supporting and regenerative, biocompatible, and biodegradable implants.





(c)

Figure 5. a) Load-Displacement curves, b) Young's Modulus, and c) Hardness of Chitosan, PLA, and PCL films and blends.

3.6 SEM and EDS Analysis.

The objective of this analysis was to validate the structural integrity, evaluate the miscibility of the polymer, and verify the absence of phase separation in the microstructure of the ternary film. As shown in figure 6, the surface and cross-sectional views of the obtained sample were compact, continuous and without any

holes or cracks indicating the homogenous composition of the CS, PLA, and PCL without any phase separation at the selected ratios. Also, the resultant morphology supports the molecular events related to the successful blending of the three polymers as discussed in the FTIR analysis. The EDS analysis investigated three different spots labeled as Top, Middle, and Bottom at the film's cross-section for weight ratios of carbon, oxygen and nitrogen. The abundance of carbon and oxygen is related to the nature of the utilized organic polymers and the nitrogen existence is specifically related to the CS. These weight ratios were compared to a theoretically perfect blend of the polymers calculated by their stoichiometry (**Table 3**). The EDS measurements were found to be in good agreement with the theoretical values, further supporting that a well-mixed polymer solution resulted in a homogenous blend.

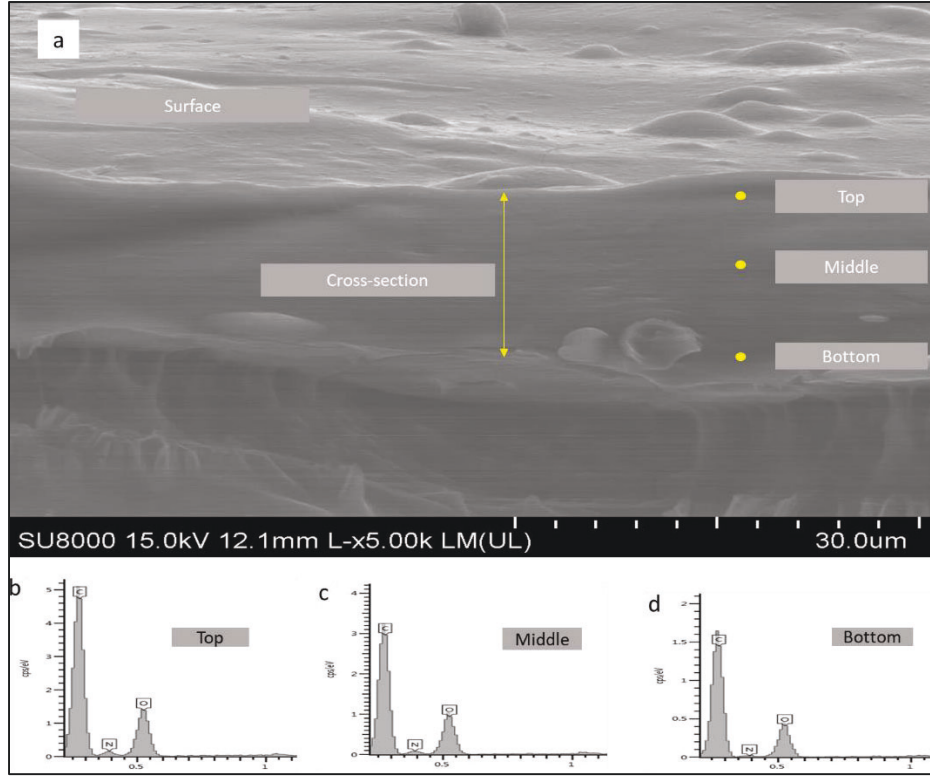


Figure 6. a) Surface and cross-sectional view of the CS-PLA-PCL thin film, b-d) Elemental composition of the top, middle, and bottom spots.

Table 3: Calculated Versus experimental elemental ratios of the ternary film obtained from EDS.

	C	N	O
Calculated Blending wt.%	60%	6%	34%
Top	63%	8%	29%
Middle	62%	7%	31%
Bottom	67%	5%	28%

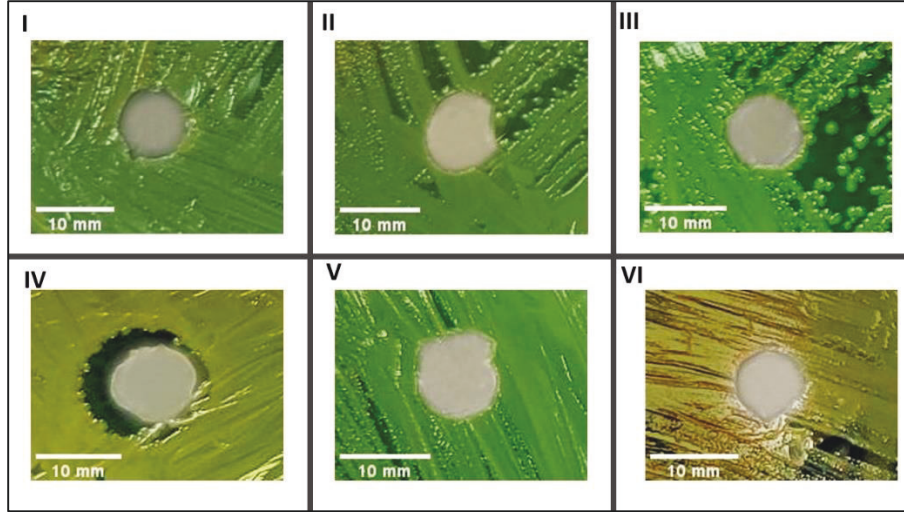
3.7 Antibacterial Test

The six prepared films exhibited remarkable efficacy on the gram-positive and gram-negative microbes (Figure 6). Placing the ternary film samples over the cultured agar dishes resulted in the film swelling, then

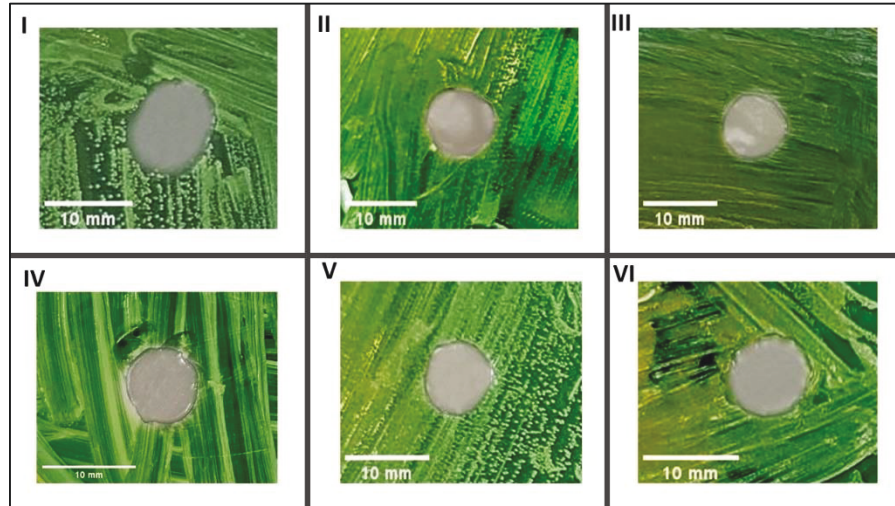
the expansion in size produced wider inhibition zones compared to the neat and the binary films. The mean diameters of the inhibition zones created by the films on the *E. coli* and *S. aureus* dishes are summarized in Table 3. The neat chitosan film yielded inhibition areas with average diameters of 8.35 mm and 9.91 for *E. coli* and *S. aureus*, respectively. The blend films also conserved their inhibition effect on the growth of the two bacteria types. For example, the diameters of the CS+ PCL and CS+ PLA blend films' inhibition zones against *E. coli* were approximately 8.89 and 8 mm, respectively, and their inhibition region against *S. aureus* reached up to 8.81 and 7.62 mm in diameter, respectively. The blend films that contained CS, PLA, and PCL caused a robust inhibition effect on the cultured agar plates: the inhibition diameter for *E. coli* was between 12.39 and 7.43, and between 7.42 and 7.7 mm for the *S. aureus* microbes. These findings are attributed to Chitosan's intrinsic antibacterial activity. Chitosan disrupts the bacterium cells by binding to their negatively charged walls and inhibiting bacteria growth⁴³. The material then binds to DNA, preventing its replication and causing cell death. Chitosan can work as a chelating agent that binds with trace metal elements, producing toxins and inhibiting bacterial proliferation⁴⁴. The ternary blended films composed of different weight ratios of PLA and PCL while the Chitosan was the same in the binary and the ternary films (67%). We expected a reduction in the antibacterial activity equivalent to the total weight ratio of PLA and PCL in binary and ternary blends (about 33%) due to poor antimicrobial properties of PLA and PCL.⁴⁵⁻⁴⁷ However, the inhibition zone in CS+PLA(33%) film for *S. aureus* was decreased by only 23% compared to Chitosan film, whereas the CS+PCL(33%) was only reduced by 11%. This trend was also obvious in ternary blends where the reduction ratio was from 22.3% to 25% at different weight ratios of PLA and PCL. These findings highlight and validate the ability of blending method to avoid the degradation of Chitosan antimicrobial activity in presence of both PLA and PCL. These results confirm the strong antimicrobial influence of blended films against positive and negative gram bacteria, which makes them suitable for packaging and medical applications.

Table 4. The diameters of the prepared films' inhibition zones against *E. coli* and *S. aureus* microbes.

Film	<i>E. coli</i> (D ± SD) mm	<i>S. aureus</i> (D ± SD) mm
CS	8.35 ± 0.39	9.91 ± 0.79
CS +PCL	8.89 ±0.90	8.81 ± 0.50
CS +PLA	8.00 ± 0.44	7.62 ± 0.27
CS +PLA+PCL	12.39 ± 0.73	7.43 ± 0.40
CS + PLA(10%)+ PCL(23%)	8.64 ±0.69	7.42 ± 0.16
CS + PLA(23%)+ PCL(10%)	7.42 ± 0.30	7.70 ± 0.22



(a)



(b)

Figure 7. Antibacterial tests against (a) *E. coli* and (b) *S. aureus* of (I) CS, (II) Cs+PCL, (III) CS+PLA, (IV) CS+PCL+PLA, (V) CS+ PLA(10%)+ PCL(23%), and (VI) CS+ PLA(23%)+ PCL(10%) films.

4 Conclusion

This work successfully developed a novel tri-biopolymer thin films of Chitosan, PLA, and PCL polymers using the solution blending method at various blending ratios. The characterization outcomes obtained by comparing the blends with neat films confirmed that the blending technique resulted in functional biopolymeric films that are consistent with enhanced physiochemical properties. The novel this films' mechanical, optical, thermal properties, and water hydrophilicity behavior were adequately optimized. All CS+PLA+PCL films demonstrated a stable blend structure with robust and effective antimicrobial effect against both gram positive and gram-negative bacteria. These interesting properties originated from the homogenous and synergetic composition of the ternary films. As a future work of our group, we will

investigate the potential of employing these films' in medical applications, including wound dressing and tissue regeneration applications.

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Credit authorship contribution statement

Ashraf Al-Gorae: Original draft, experimental work, data collection and analysis. **Ali S. Alshami:** Conception, review and editing, Supervision, Fund acquisition. **Abdulrahman Al-Shami:** Original draft, experimental work, data collection and analysis. **Jason Power:** Experimental work, data collection and analysis. **Jadyn Guidinger:** Experimental work, review & editing. **Christopher Buelke:** Experimental work, data collection and analysis. **Musabbir Jahan Talukder:** Data collection and analysis. **Xiaodong Hou:** Review and editing.

Data Availability: The datasets generated and analyzed during the current study can be obtained from the corresponding author upon reasonable request.

Declarations:

Conflicts of Interest: The authors state that they do not have any known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Supplementary information: Not applicable.

Ethical approval: Not applicable.

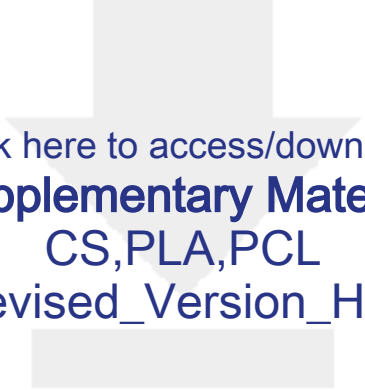
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