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Preparing and Characterizing Novel Biodegradable Starch/PVA-Based Films with Nano-Sized Zinc-Oxide Particles for Wound-Dressing Applications

Mohammad Mohsen Delavari * and Ion Stiharu

Department of Mechanical, Industrial and Aerospace Engineering, Concordia University, Montreal, QC H3G 1M8, Canada; ion.stiharu@concordia.ca

* Correspondence: mohammadmohsen.delavari@concordia.ca

Abstract: Given recent worldwide environmental concerns, biodegradability, antibacterial activity, and healing properties around the wound area are vital features that should be taken into consideration while preparing biomedical materials such as wound dressings. Some of the available wound dressings present some major disadvantages. For example, low water vapor transmission rate (WVTR), inadequate exudates absorption, and the complex and high environmental cost of the disposal/recycling processes represent such drawbacks. In this paper, starch/polyvinyl alcohol (PVA) material with inserted nano-sized zinc-oxide particles (nZnO) (average size $\leq$ 100 nm) was made and altered using citric acid (CA). Both ensure an efficient antibacterial environment for wound-dressing materials. The film properties were assessed by UV–Vis spectrometry and were validated against the UV light transmission percentage of the starch/ polyvinyl alcohol (PVA)/ zinc-oxide nanoparticles (nZnO) composites. Analyses were conducted using X-ray Spectroscopy (EDX) and scanning electron microscopy (SEM) to investigate the structure and surface morphology of the materials. Moreover, to validate an ideal moisture content around the wound area, which is necessary for an optimum wound-healing process, the water vapor transmission rate of the film was measured. The new starch-based materials exhibited suitable physical and chemical properties, including solubility, gel fraction, fluid absorption, biodegradability, surface morphology (scanning electron microscopy imaging), and mechanical properties. Additionally, the pH level of the starch-based/nZnO film was measured to study the prospect of bacterial growth on this wound-dressing material. Furthermore, the in vitro antibacterial activity demonstrated that the dressings material effectively inhibited the growth and penetration of bacteria (*Escherichia coli*, *Staphylococcus aureus*).

Keywords: starch-based films; biodegradable; polyvinyl alcohol; nano-zinc-oxide; wound dressing; characterization

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1. Introduction

These days, given the current worldwide ecological-related circumstances, social concerns, and growing environmental awareness, new efforts have been initiated in a variety of industries to develop more sustainable new products and processes suitable to the environment. The concerning reasons such as lightweight, convenience, safety, low price, and sounding appealing, which all contributed to the rapid growth in the use of petroleum-derived materials such as polystyrene, polyvinyl chloride, and nylon, with such materials used as biomedical fabrics or for packaging in industrial, and agriculture applications [1], have left a considerable footprint within the environment. A recent study shows that around 8300 million metric tons represent the total amount of waste produced, and the resulting plastic waste is estimated at 6300 million metric tons, of which only 9% has been recycled and 12% is incinerated [2]. Hence, many previous investigations [3–7] have shown interest in tackling these environmental issues by replacing conventional plastic materials with more eco-friendly fabrics for the abovementioned applications.

As is stated above, one of the areas that recently has attracted attention is wound dressing products. Since the skin is the first protective and the largest organ that shields human internal organs from possible hazards outside the person's body, the risk of damage is substantially higher than any other internal organ because it is unprotected and it can break down relatively effortlessly, causing varieties of chronic non-healing wounds [8]. Worldwide, the wound products annual cost for all sorts of wounds has been predicted to be USD 96.8 billion [9]. In 2020, due to the coronavirus disease (COVID-19) pandemic, health care, including wound care, was noticeably disrupted worldwide and emphasized the need for a universal approach to resolving this obstacle [9]. Therefore, the use of biodegradable materials such as starch and polyvinyl alcohol could represent a suitable response to the environmental challenges sparked by synthetic polymers such as nylon and the expansion in the biomedical market requirements.

Starch is native, renewable, plentiful, low-priced, biodegradable, and plant-based (corn, wheat grains, potato, and rice) [10], attracting considerable attention to substitute conventional materials. Starch is a unique carbohydrate made of individual soluble granules in water [11]. However, its applications are limited by starch's poor mechanical properties, high brittleness, and reduced water sensitivity. Mainly, adding up other macromolecular compounds into starch can improve the physicochemical properties of the films [12]. Among them, a biodegradable and water-soluble synthetic polymer, polyvinyl alcohol (PVA), is frequently mixed with starch to enhance its properties. PVA also has oil, grease, and solvent resistance. PVA has no odor and is non-toxic. It shows good mechanical properties such as high tensile strength and elongation-at-break. Compared to starch properties, PVA/starch materials possess better degradation, strength, and flexibility characteristics. On the other hand, both polymers are not compatible with one another; therefore, glycerol (GLY) and citric acid (CA) could be added to improve the mechanical properties of the PVA/starch films as plasticizers [10,13,14]. Citric Acid (CA) exhibits high acidity; as a result, including citric acid in the solution of the film enhances the antibacterial properties [4,15,16], which represents one of the critical requirements for wound dressings. Moreover, compared to glycerol, citric acid carboxyl groups have been identified to form stronger hydrogen bonds with starch-based/polyvinyl alcohol composite films [15].

In addition, embedding non-toxic metal-oxide particles such as nano-zinc-oxide (nZnO) for its ability to enhance the thermal, mechanical [17], and vapor transmission rate properties in wound dressings [18–20] and packaging material applications has attracted a great deal of attention and has been investigated in several publications [21–23]. Incorporating the nZnO into wound dressing sheets supports the antibacterial features [24,25] and increases healing rates [26]. Additionally, ZnO is acknowledged as safe, and according to the Food and Drug Administration (FDA) [27], it is essential for human health and physiological activity that each individual takes in 10 mg of zinc per day [28], and the cytotoxicity analysis of the nZnO particles has proved that a concentration of 40 μg/mL or less could be considered non-toxic [29].

Although a few research groups have used nZnO particles in different applications such as bioplastics, wound dressings, and food packaging [17–23], based on our best knowledge, the effects of adding nZnO particles on functional starch/PVA/glycerol-based wound dressings have not been studied. Therefore, in this work, the nZnO particles were inserted into a starch/PVA/CA/glycerol film (SPCG) to prepare biodegradable and antibacterial wound dressing materials. To establish the proper wound environment for the ideal wound-healing procedure, the material water vapor transmission rate (WVTR), swelling index, antibacterial characteristics, biodegradability, gel fraction, solubility, mechanical properties, and antibacterial activity were assessed and validated. Then, the interactions between the nZnO particles and starch-based blends, which lead to UV and visible light transmittance, were evaluated using the UV/Vis spectrometry device. The wound dressing material morphology properties were also examined utilizing SEM imaging.

2. Materials and Methods

Potato starch (molecular weight is 342.30 g/mol, the average granule size of starch is 80 µm), polyvinyl alcohol (PVA, molecular weight 16,000–23,000 g/mol and 88% hydrolyzed), and citric acid monohydrate (molar mass, M = 192.12 g/mol; assay ≥ 99%) were obtained from Sigma-Aldrich, Oakville, ON, Canada. Glycerol (M = 92.05 g/mol; purity (GC) ≥ 99.0%) was acquired from Fisher Scientific, Hampton, NH, USA. The ZnO nanoparticle (81.39 g/mol) with average dimensions of ≤100 nm, Penicillin G sodium salt (Sigma 13752), *Staphylococcus aureus* (*S. aureus* S2014), and *Escherichia coli* (*E. coli* EC1) were purchased from Sigma Aldrich, Cleveland, OH 44125, USA. DI water equipment provided the deionized water (DI) in our laboratory (MIAE Department) at Concordia University.

2.1. Blends Preparation Procedure

The process was initiated by solving 2.5 g of polyvinyl alcohol in 50 mL of DI water on a magnetic stirring hotplate (Figure 1a). The solution was heated, maintained around 85 °C, and stirred at 300 rpm to prevent bubbles and foam. In 15 min, the PVA granular was completely dissolved in water. Then, 2.5 g of potato starch and the ZnO nanoparticle powder (0.2, 0.25, 0.3, and 0.35 g) were mixed in DI water homogenously utilizing an ultrasonic mixer for 10 min (Figure 1b), from which process a solution without bubbles was produced (Figure S1).

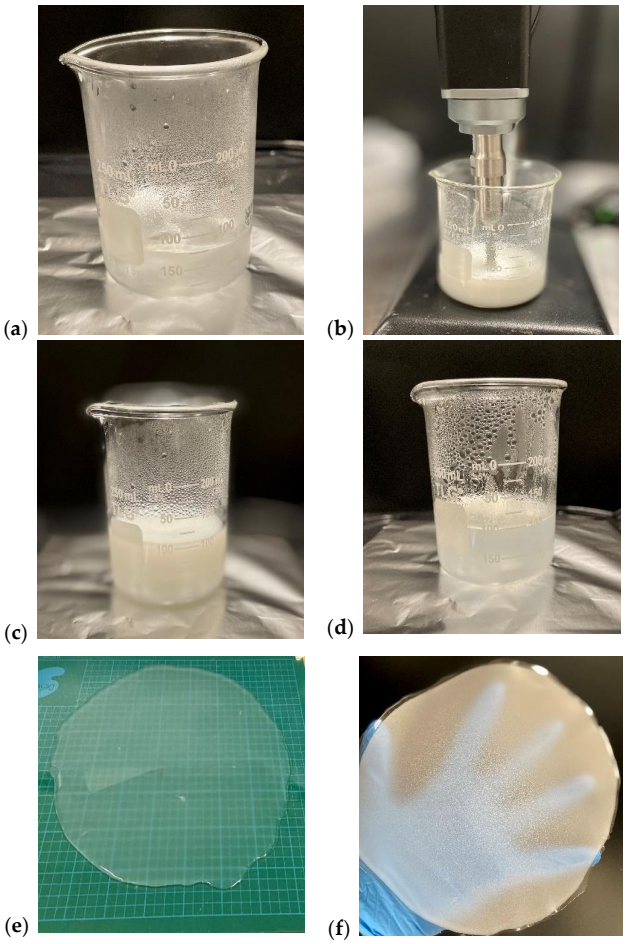


Figure 1. The blend-preparation steps: (**a**) dissolving PVA in DI water, (**b**) mixing starch and nZnO powder, (**c**) adding starch/nZnO and PVA solutions, (**d**) after adding glycerol and citric acid at the interval of 10 min, (**e**) pouring the final solution on the flat glass, (**f**) starch-based polyvinyl alcohol (PVA) blend with inserted nano-zinc-oxide particles (nZnO).

By adding the starch and nZnO solution to the PVA solution and keeping the same stirring speed and the solution temperature, a viscous solution was prepared after 15 min (Figure 1c). Glycerol (2 g) and citric acid (0.5 g) were added to the solution one at a time at the interval of 10 min for each material (Figure 1d). The content for each sample is provided in Table 1. Later, the final viscous blend was poured on clean flat glass and kept at room temperature (Figure 1e). Then again, the mixture should be warmed up for 30 min at 95 °C after 24 h of drying in the ambient condition. Finally, the starch/polyvinyl alcohol films (S1–S4), which embed nZnO particles, could easily be separated from the surface (Figure 1f). Dressings were then placed in dry and air-free containers to prevent humidity absorption and reduce the chance of contamination. After producing the starch/polyvinyl alcohol films, the following experiments were conducted to validate the wound dressing characteristics.

Table 1. Starch-based-material contents.

	Starch (g)	PVA (g)	Citric Acid (g)	Glycerol (g)	nZnO (g)
SPCG	2.5	2.5	0.5	2	-
S1	2.5	2.5	0.5	2	0.2
S2	2.5	2.5	0.5	2	0.25
S3	2.5	2.5	0.5	2	0.3
S4	2.5	2.5	0.5	2	0.35

2.2. Water Vapor Transmission Rate (WVTR)

A few ambient parameters such as moisture and oxygen are essential [30] in order to yield faster wound healing. Therefore, the standard ASTM E398 method was used to measure the water vapor transmission rate (WVTR). The samples (S1–S4) were cut in a circular shape (60 mm in diameter), following procedures outlined by Delavari and Stiharu [20] during the data collection.

2.3. UV–Vis and IR Spectroscopy

For validating the dressing light absorption/transmission, the samples (S1–S4) were cut into appropriate shapes and positioned in a UV–Vis spectrophotometer (LABOMED INC. UV-2550), following the procedure outlined in our previous work [20]. The light transmittance properties of the film samples (S1–S4) were determined within the 250 nm to 1000 nm span.

2.4. Swelling Index

The swelling index needs to be assessed to investigate the fluid absorption ability of the films (S1–S4). This approach starts with dipping the samples (1×1 cm^2) into phosphate-buffered saline (PBS) solution followed by incubation at 37 °C for 24 h; the swelling index percentage is calculated through Equation (1) [4], where W_0 and W_s are the dry weight and wet weight after dipping sample in PBS, respectively. The average values for five specimens were reported.

$$\text{S.I.} = \frac{W_s}{W_0} \times 100\% \quad (1)$$

2.5. Solubility and Gel Fraction

The starch/polyvinyl alcohol/citric acid/glycerol/nZnO films (1×1 cm^2) were dipped in DI water for 24 h, following procedures outlined by Delavari and Stiharu [20]. The samples' (S1–S4) solubility and gel fraction were assessed (five specimens were used, and the average values were reported) using Equations (2) and (3), where W_0 is the sample dry weight, and W_d is the final drying weight (at 37 °C) after dipping in DI water.

$$\text{S} = \frac{W_0 - W_d}{W_0} \times 100\% \quad (2)$$

$$\text{GF} = \frac{W_d}{W_0} \times 100\% \quad (3)$$

2.6. Weight Loss

The wound-healing rate and efficiency count on many factors, such as the degradation of the dressing materials. This aspect helps the appropriate drug to be released into the wound area. To evaluate the materials' (S1–S4) weight loss percentage after submerging them in PBS solution with 7.4 pH at 37 °C for 96 h. Equation (4) defines the weight loss percentage [4], and W_0 and W_f describe the sample dry weight before submerging in the PBS media and the weight of the pieces after drying at 37 °C, respectively. On five specimens, average values were calculated and reported.

$$\text{Weight Loss \%} = \frac{W_0 - W_f}{W_0} \times 100\% \quad (4)$$

2.7. Mechanical Properties

The ultimate tensile strength and elongation-at-break were measured and analyzed for the samples (S1–S4); five specimens were used, and the average values were reported. These experiments were conducted by utilizing a unique high-resolution testing device built in-house by our team, which was used in our recent article [20] for wound-dressing and delicate-film testing. According to the ASTM D882-10 test, the tensile test should be completed under ambient conditions. The thickness (t) of the samples (S1–S4) was measured from five points, and the average values were used in the calculations for each of the samples. The results are shown in (Figure 2).

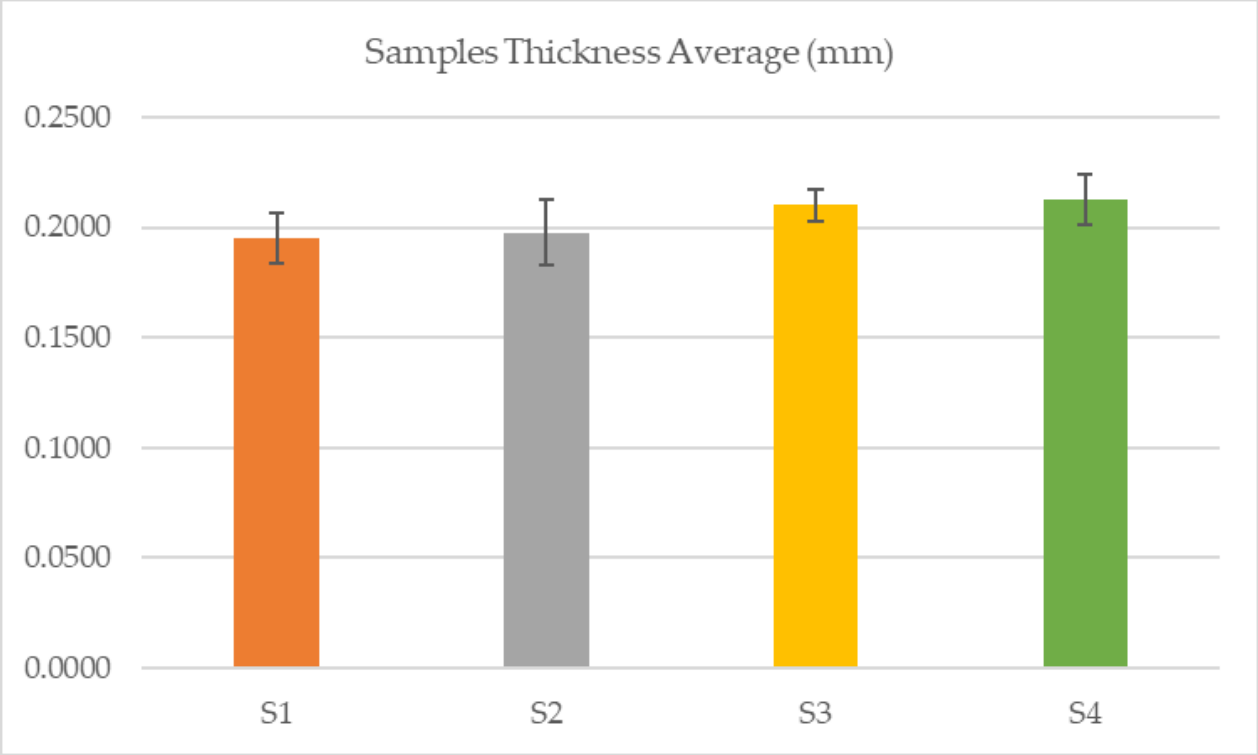


Figure 2. The average thickness of the samples (mm).

2.8. The pH Levels

The proper wound-healing procedure requires various parameters, and one of those essential factors is the pH value [31]. A calibrated OMEGA waterproof pH tester, model PHH-7011, was utilized to measure the dressing samples' (S1–S4) pH level [20].

2.9. The Scanning Electron Microscopy and X-ray Spectroscopy

The films' (S1–S4) morphological properties were examined using Scanning Electron Microscopy (SEM, Hitachi S 3400N) at an accelerating voltage of 15 kV. Elemental analysis of the dressings (S1–S4) was conducted using SEM by energy-dispersive X-ray spectroscopy (EDX). A 1.5 × 1.5 cm^2 of each film samples was adhered to a carbon tape attached to the metallic stubs.

2.10. The Antibacterial Activity

The antibacterial activity of PVA/St/nZnO composites against *E. coli* (gram-negative) and *S. aureus* (gram-positive) was investigated according to the agar diffusion method [6]. The PVA/St/nZnO samples (S1–S4) were subjected to bacteria in a solid environment (nutrient agar). The inhibition zone around each sample was considered the antibacterial property of nZnO starch-based materials. The prepared bacterial suspension was spread on an agar plate, and then the films (S1–S4) were placed on the agar plate. These plates were kept in an incubator at 37 °C for 48 h.

3. Results

3.1. Water Vapor Transmission Rate (WVTR)

In an ideal wound dressing, the WVTR should be between 2000 and 2600 g/m^2/day in order to avoid dryness and moisture loss, which could result in bacterial infections [32]. However, depending on the type of skin wound, the WVTR may need to be higher; for illustration, in order to prevent granulating wounds, first-degree burns require WVTR of 3000 g/m^2/day, whereas severe burns require 5000 g/m^2/day [32,33]. After combining the nZnO particles with the PVA/starch blend (SPGC), the new solutions' (S1–S4) WVTRs were recorded from 2691 g/m^2/day to 2728 g/m^2/day (Figure 3). When compared to the SPGC blend, there was a reduction in WVTR from 4146 to an average of 2704 g/m^2/day. Therefore, the measured WVTR demonstrates that the prepared material could be used as an ideal wound dressing.

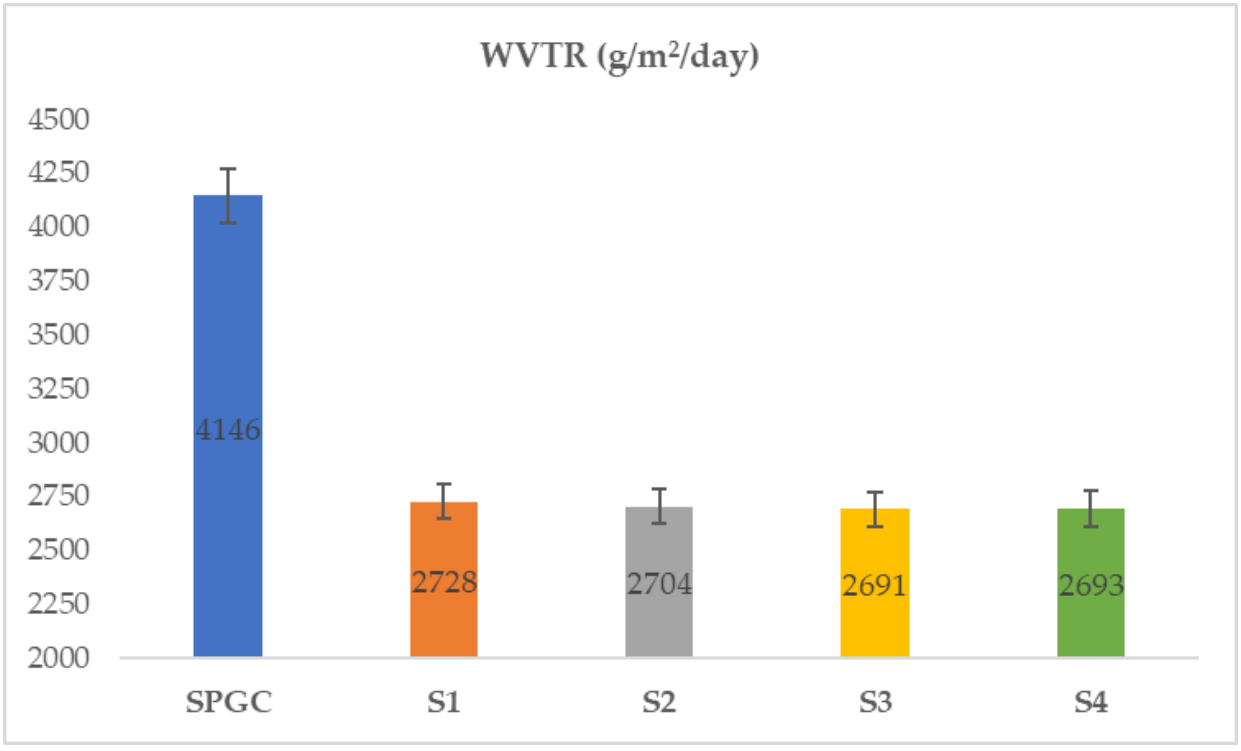


Figure 3. Biodegradable starch/PVA wound dressings' WVTR before (SPGC) and after adding nano-ZnO particles (S1–S4) to the starch-based/polyvinyl alcohol composite.

3.2. UV–Visible Spectroscopy

The reflective properties of the starch/PVA-based samples (SPGC and S1–S4) confirmed the presence of the nZnO particles. Figure 4 illustrates that having nZnO in the material triggered a decrease in the average transmittance percentage of the material from 70 percent to an average of 30 percent, meaning that less UV light can pass through the material due to the intense UV light absorption or scattering properties of nZnO. The highest UV light transmission was related to the SPGC sample without nZnO particles.

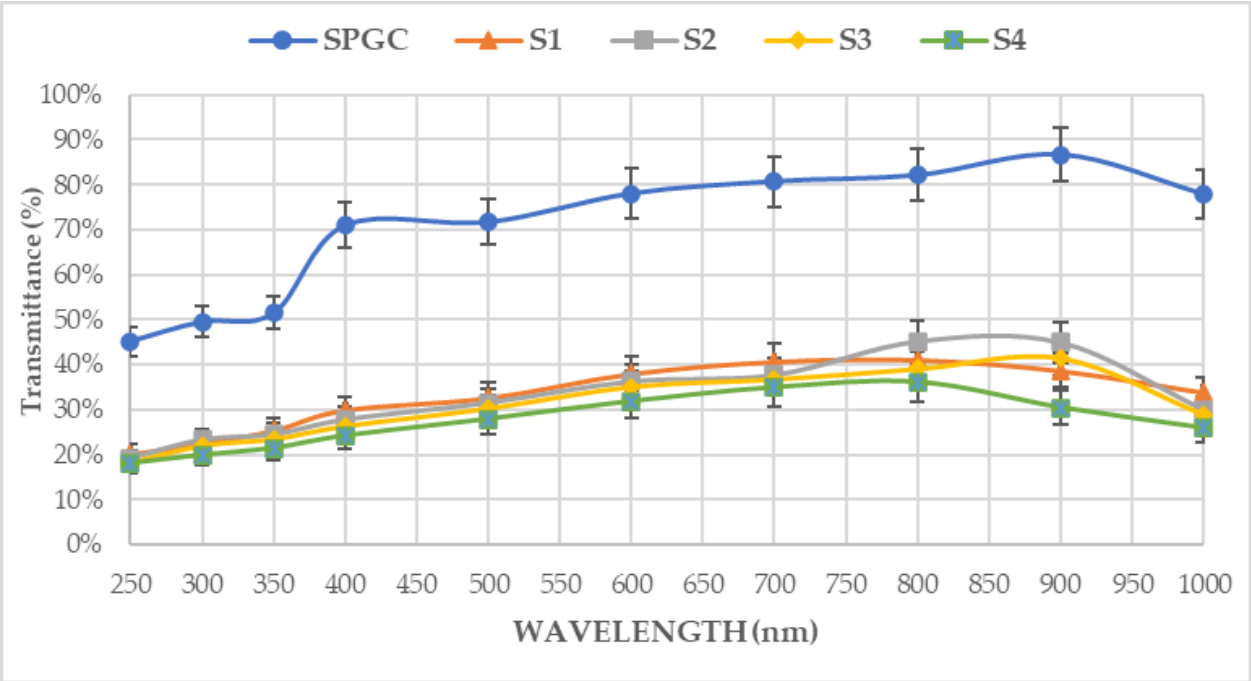


Figure 4. UV–Vis transmittance percentage of biodegradable starch/polyvinyl alcohol wound dressings before (SPGC) and after adding four different amounts of nano-sized ZnO particles to the material (S1–S4).

3.3. Starch-Based Films' Swelling Index

Figure 5 illustrates the effects of adding the nano-ZnO particles to the starch/polyvinyl alcohol/citric acid/glycerol film (SPGC) solution on the swelling index. The swelling index of the new composites (S1–S4) slightly decreased from 492 to 402 percent at a relatively consistent crosslinking temperature of 85 °C and 0.5 g of CA concentration. Moreover, there was a slight decrease in this blend index compared to the sample without nano-sized ZnO particles (SPGC). These results were consistent with other studies [34,35] and could be explained by the tortuous pathways created, which might have favored the narrowing of pore channels, and due to the increased diffusion paths of the polymer matrix, which led to an improved water barrier property. Wound dressing materials with a much higher swelling index are equipped to absorb wound bed fluids effectively. The crosslinking temperature of 85 °C leads to a minimum of 300 percent of the swelling index, which is the least required amount in the super-absorbent wound-dressing films category [36].

3.4. Solubility and Gel Fraction

The changes in starch/polyvinyl alcohol/citric acid/glycerol film solubility before (SPGC) and after adding the nZnO particles into the solution (S1–S4) are shown in Figure 6. As validated, at the crosslinking temperature of 85 °C, the SPGC solubility fell from 58.1% to 45.3%, 45.6%, 45.4%, and 45.1% for S1 to S4 samples, respectively, after adding the nZnO to the SPGC solution. The water barrier properties of the wound dressing are improved due to the following reasons.

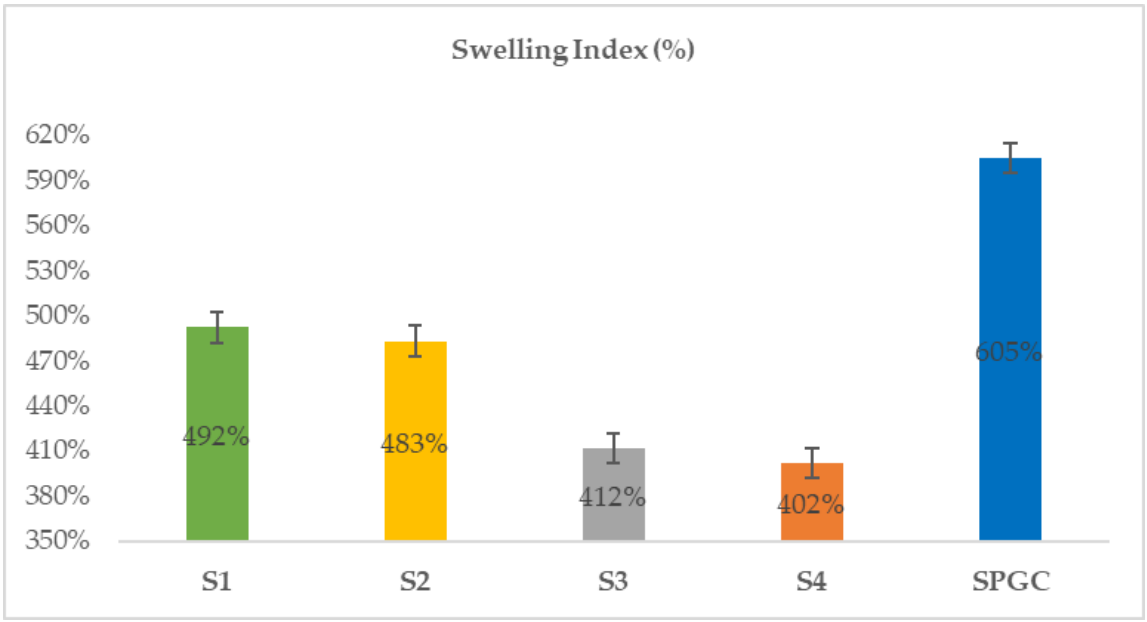


Figure 5. The outcomes of the nZnO particles on the starch-based films' (S1–S4) swelling index compared to the SPGC sample without nZnO.

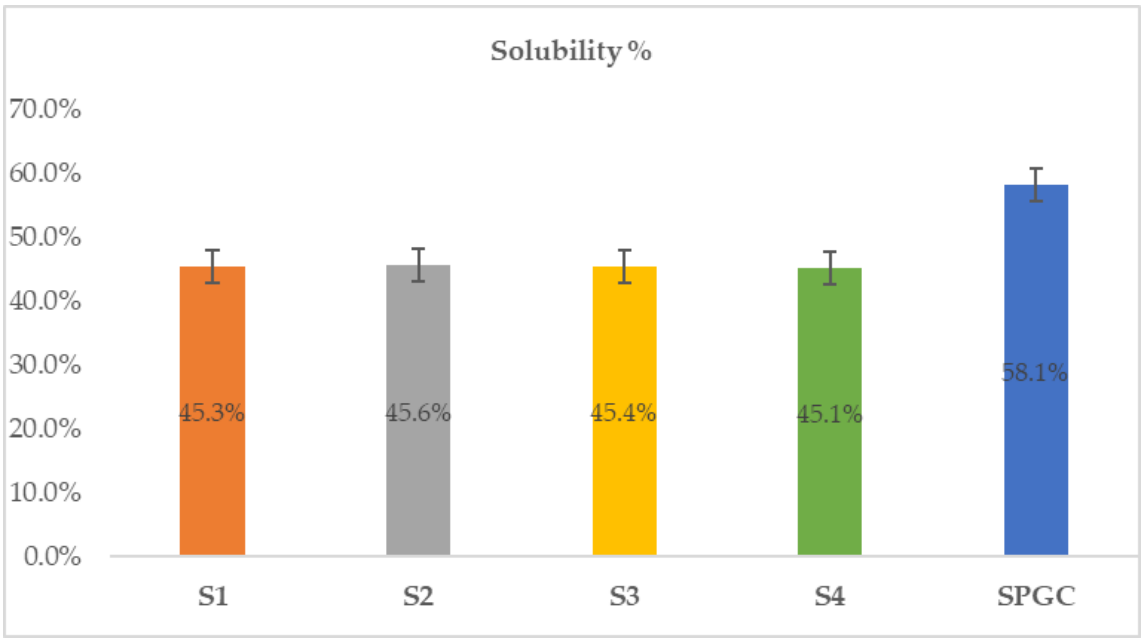


Figure 6. The solubility percentage of the starch-based films (S1–S4) was affected by adding the nZnO particles.

To begin with, this decreased solubility in the new composites (S1–S4) is feasibly caused by the reactions between nZnO particles and starch in the film structure. Many studies [37–39] have shown that the more hydrogen bonds between nanoparticles and other compounds in the composite matrix, the less free water molecules can interact with such films embedded with nZnO particles.

Furthermore, the intermixed crosslinking and plasticizing influences led to a decrease in the solubility percentage. In fact, the crosslinking effect reduces the solvent transportation into the polymer structure, reducing starch-based solubility. The other studies [35] reported the same observation and results, and it occurs due to the closing of pore channels and the

increase in the polymer matrix diffusion path length that were both initiated by adding the nZnO particles to the blend.

Figure 7 illustrates starch-based films' gel fraction percentage reached 70.3% from 65.2%, adding the nano-ZnO particles to the starch/PVA/CA/glycerol film (SPGC) solution at the steady 85 °C crosslinking temperature. This feature demonstrates how polymer molecule chains were created and gel fraction correlated with the films' mechanical strength. To be more specific regarding this correlation, the polymer crosslinking ability will be enhanced as the gel fraction increases, and thus the mechanical strength will increase. Even though adding CA within the formulation increases the free residual, it has plasticizing and crosslinking impacts on the final material polymer networks.

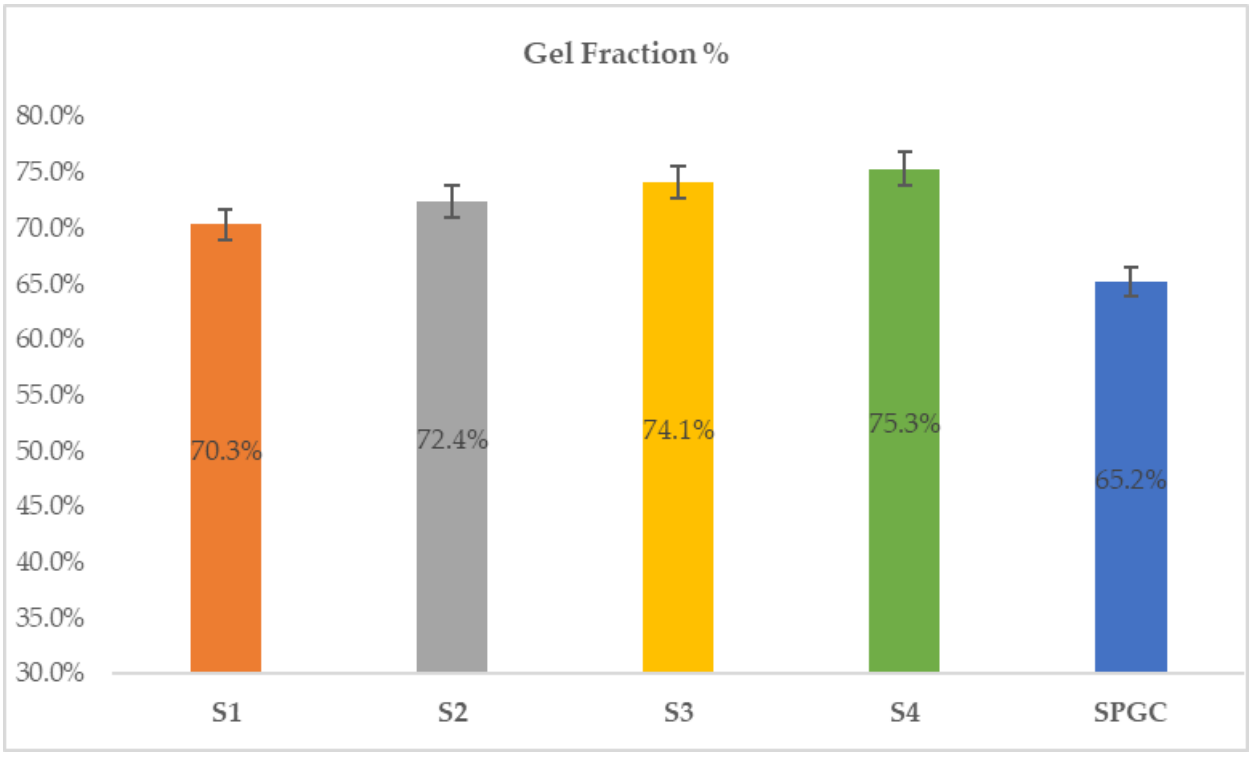


Figure 7. Comparison of the starch-based film (SPGC) and the samples (S1–S4) with nZnO gel fraction percentage.

3.5. Weight Loss

The starch/polyvinyl alcohol/citric acid/glycerol films' (S1–S4) weight loss with and without the nZnO particles was measured regarding film degradation in phosphate-buffered saline (7.4 pH) for the period of 96 h incubation at 37 °C. The SPGC sample weight loss reduced marginally from 58.2% without the ZnO to 49.8%, 48.3%, 47.2%, and 46.9% for S1, S2, S3, and S4 samples, respectively, after adding the nZnO particles (S1–S4) to the material blend (Figure 8).

The starch/polyvinyl alcohol/nZnO wound dressings' (S1–S4) degradation properties may be altered by the citric acid concentrations and crosslinking temperature, and the results approve the solubility data as they refer to biodegradability enhancement of such blends or materials. The wound exudate partially dissolves these films and informs a hydrophilic wound dressing that keeps the wound safe from infections and encourages granulation [40]. In order to guarantee wound dressing materials' technical feasibility, accurate degradation characteristics need to be applied to these dressings; therefore, the material should be porous and enable higher water saturation to develop higher weight-loss levels.

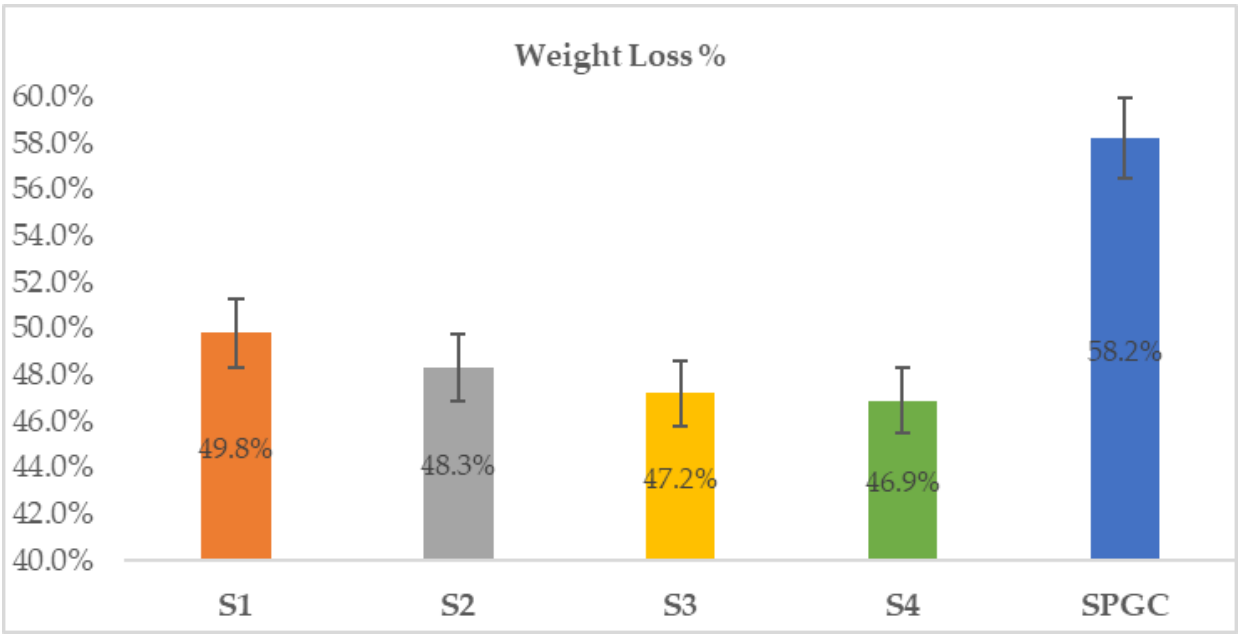


Figure 8. Weight-loss percentage comparison of the starch-based film (SPGC) and the samples (S1–S4) with nZnO.

3.6. Mechanical Strength

To study wound dressings' ultimate tensile strength (UTS) and elongation-at-break were gathered as essential factors for biomedical dressings. Many aspects, such as the temperature, and physical and chemical conditions, could influence the mechanical properties of the films [25].

The results indicate that the materials' (S1–S4) UTS changes from 6.14 (without nZnO) to 15.33, 16.61, 15.94, and 16.31 MPa, respectively, after adding ZnO nanoparticles to the starch-based/polyvinyl alcohol/citric acid/glycerol solution (SPGC), as shown in Figure 9. There are no remarkable changes in the ultimate tensile strength after increasing the nZnO content in the solutions (S1–S4). The film elongation-at-break (Figure 10) decreased gradually as the ZnO nanoparticles were added to the solution. The increase in the tensile strength of the films could be due to the uniform dispersion of the ZnO nanoparticles in the composite matrix since their presence creates unique surface properties. In addition, the moisture content of the material directly influences the film's mechanical properties. By adding nZnO particles to the solution, the free water molecules content decreases, which reduces flexibility and increases the strength of the resulting films. The reported observations are in good agreement with open literature [12,17,25]. Since the average thicknesses of the different samples used in calculating the UTS were relatively close, the effect of sample thickness on the dressings' UTS is negligible in this study.

3.7. The pH Levels

Based on the existing literature, the skin surface typically has a pH value between 4.5 and 6, which is inadequate to prevent the growth of bacteria. For example, human skin has a considerable number of *Staphylococcus aureus* on its surface, which might worsen a patient's condition with eczema disease. The pH values from 4.5 to 9.0 may well assist in reproducing such bacteria [31]. Hence, having an acidic environment will essentially help to adjust the antibacterial activities around the wound bed. The starch-based/ZnO solutions' (S1–S4) pH level was 3.91, 3.89, 3.96, and 3.94, respectively, at 37 °C. Hence, these materials can offer an adequate pH level to inhibit the growth of varieties of bacteria around the wound bed.

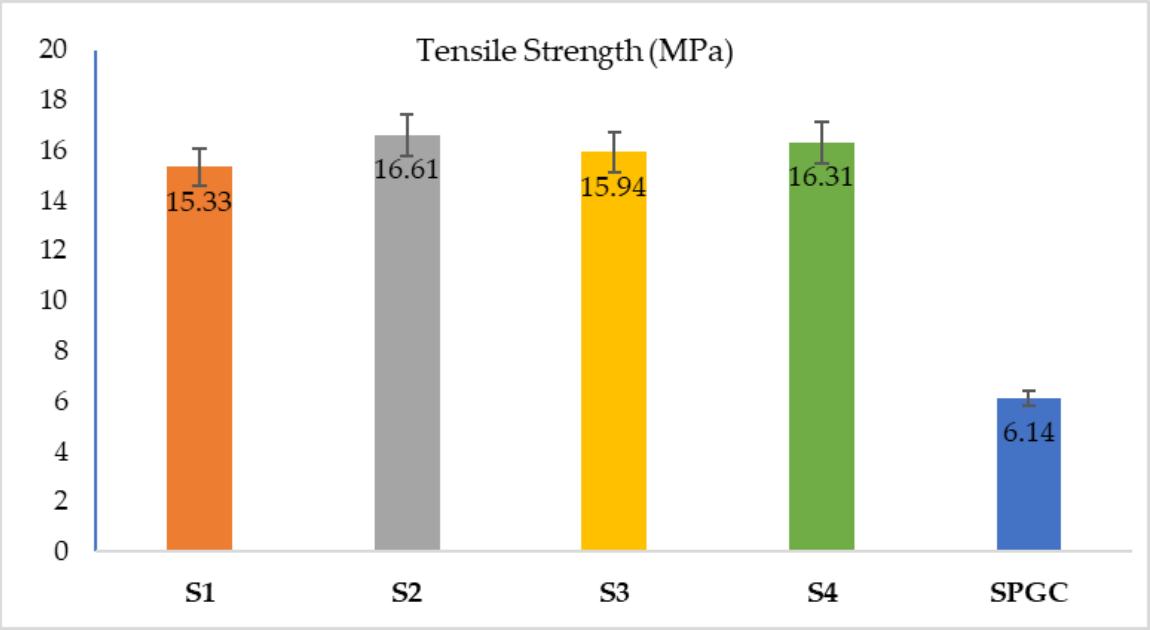


Figure 9. Wound dressings' ultimate tensile strength (UTS).

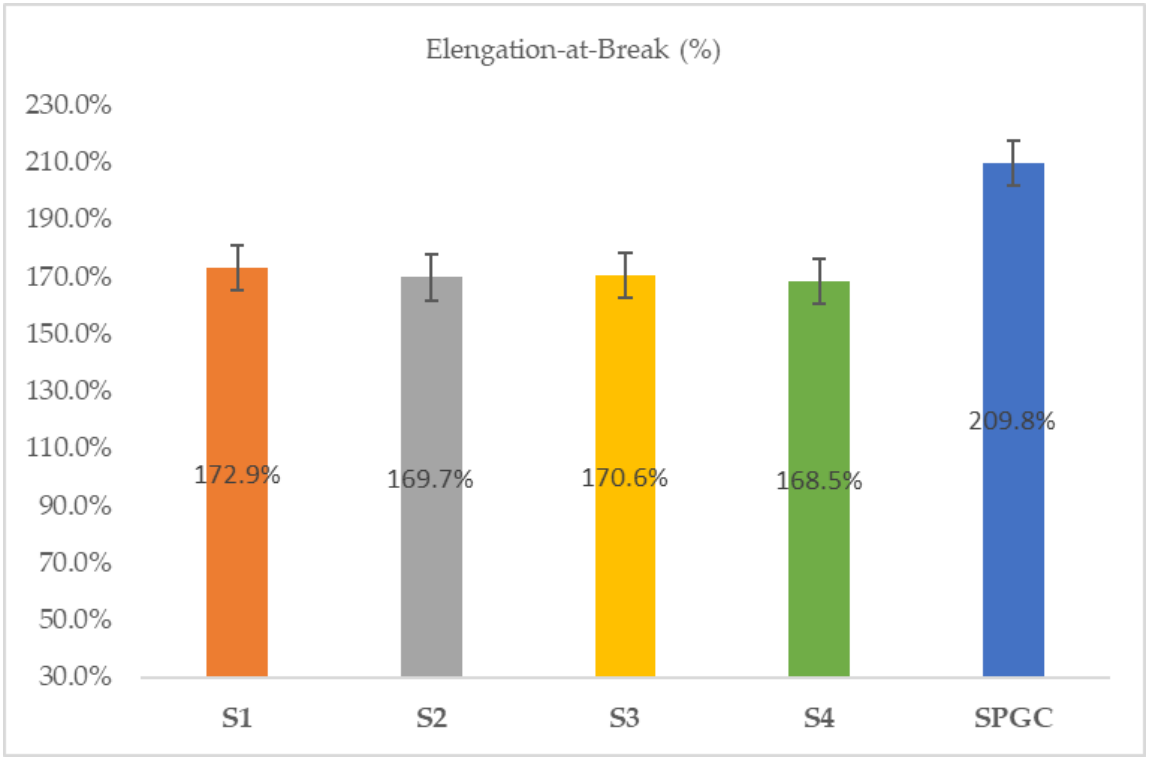
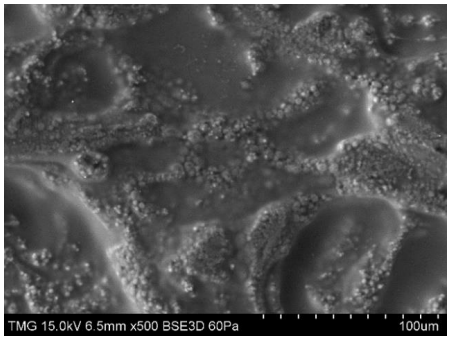


Figure 10. Effect of nZnO content on the elongation-at-break of the wound dressings.

3.8. SEM and X-ray Spectroscopy Measurements

Using SEM, the surface morphology of the films was examined (Figure 11). The starch-based/polyvinyl alcohol/citric acid/glycerol (SPGC) film exhibited a homogenous, clear, and porous surface (Figure 11a), whereas the incorporation of nZnO particles made the starch film have a rough surface (Figure 11b–e). When nZnO is dispersed in the gap between starch and PVA, it changes the molecular arrangement and the interaction between the two components. From the SEM representation of the composite film, it is clearly evident that the ZnO nanoparticles were dispersed in a matrix of PVA/starch with some degree

of agglomeration at ×500 magnification (Figure 11b–e). Clusters of nZnO, white spots (Figure 11e, the glass-side surface of the material), might be agglomerated during bioplastic formation because these spots were not found in bioplastics without nZnO.



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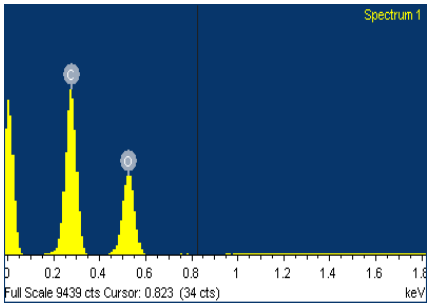

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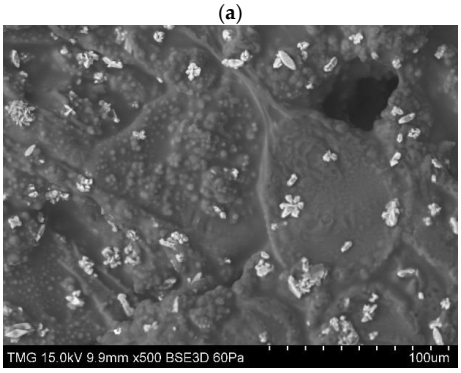

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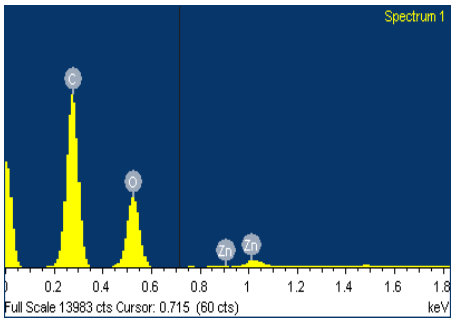
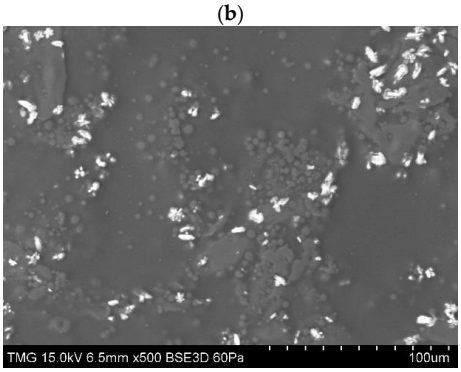
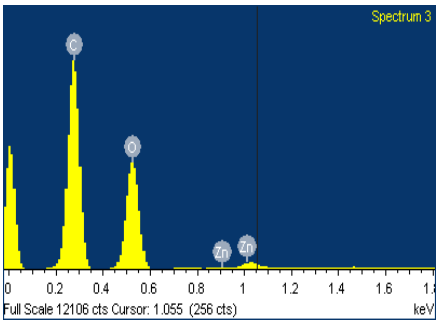


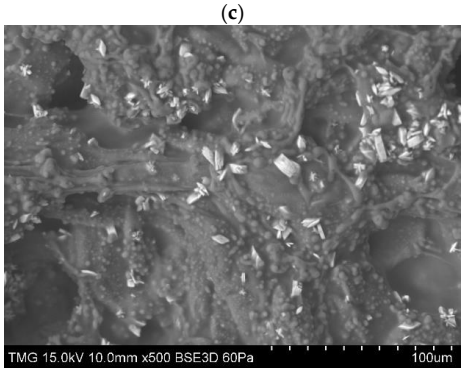
(**B**)



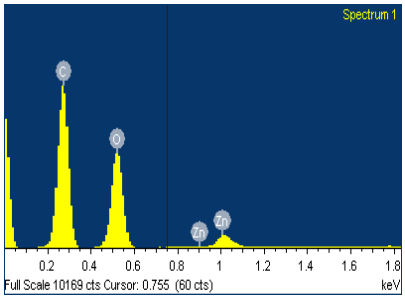
(**c**)



(**C**)

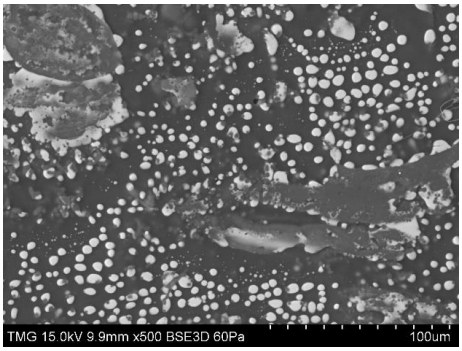


(**d**)

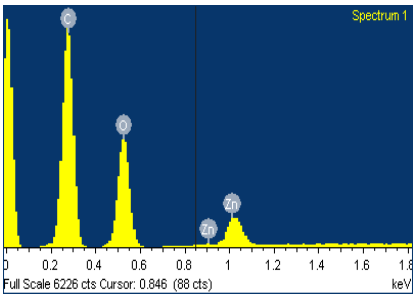


(**D**)

Figure 11. *Cont.*



(**e**)



(**E**)

Figure 11. EDX spectra (**A**–**E**) and SEM images of (**a**) starch/polyvinyl alcohol/citric acid/glycerol (SPGC), and starch/polyvinyl alcohol/citric acid/glycerol/nZnO films, (**b**) 0.2 g nZnO (S1), (**c**) 0.25 g nZnO (S2), (**d**) 0.3 g nZnO (S3), (**e**) 0.35 g nZnO (S4).

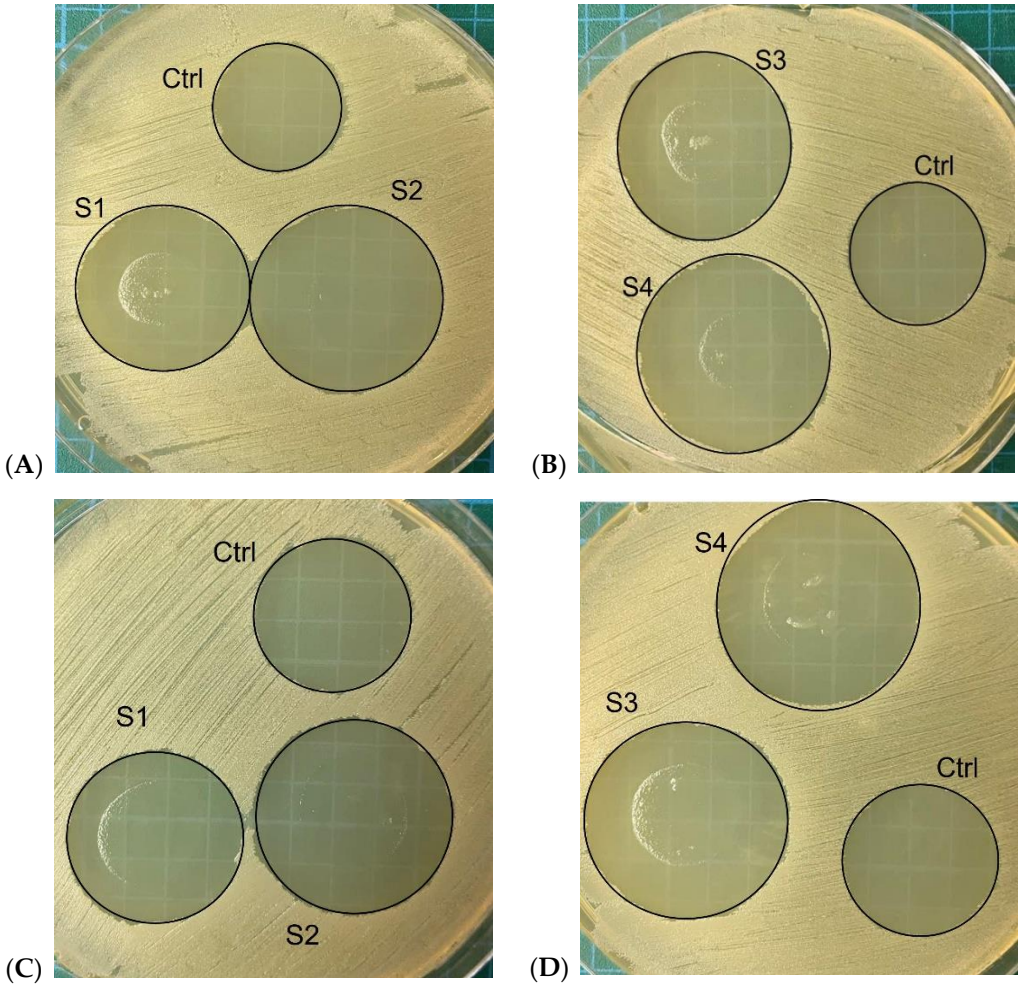
To validate the presence of zinc-oxide nanoparticles within the wound dressing, EDX analysis was performed on PVA/starch/nZnO composites, and according to the results, the content percentage of nZnO particles increased per chosen spectrum for each of the S1–S4 samples (Figure 11B–E).

3.9. Antibacterial-Property Evaluation

E. coli and *S. aureus*, two bacterial species commonly found on the skin hence, in wounds, were used to evaluate the antibacterial activities of starch-based wound dressings. Infections caused by these bacteria can produce severe skin and other soft tissue infections and delay the healing chronic and acute wounds [41]. According to published literature, nZnO particles interact with bacterial cell membranes by means of their Zn^{2+} molecule and disturb the electrical balance of bacterial cell walls [42]. Upon membrane damage, DNA and RNA leak out into the extracellular matrix (ECM) along with other cytoplasmic fluids until the cells die [43]. Accordingly, there was no inhibition zone for S1–S4 after 48 h (Figure 12), and when nZnO particles were increased, samples containing nZnO showed significant antibacterial properties.

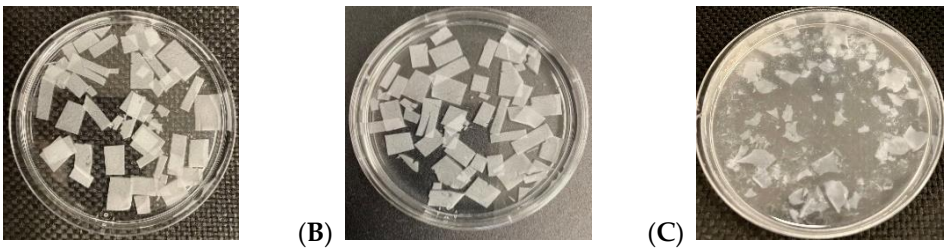
3.10. Biodegradability Analysis

Figure 13 illustrates the biodegradability properties of the starch-based materials developed in this paper by immersing samples (S1–S4) in water over a period of three weeks. Tap water was used to test biodegradation characteristics rather than deionized water. Figure 13A illustrates the immersion of the samples in a Petri dish. In Figure 13B, the starch paper did not seem to change significantly after 72 h of dipping (morphological deformations or breakage). However, after fourteen days of dipping, as shown in Figure 13C, the starch-based dressing showed signs of scattering. It was noticed here that the water was not clean. As the water was poured off, the samples of the wound dressing were found to be completely dissolved and dispersed. After twenty days of examination, the dressing did not retain its shape, and full biodegradation was confirmed. As a result of these studies, it can be concluded that starch/polyvinyl alcohol/nZnO wound dressings are biodegradable alternatives to conventional plastics that are difficult to recycle and do not decompose readily.



(**A**) (**B**) (**C**) (**D**)

Figure 12. Antibacterial activity test of starch-based materials against *E. coli* and *S. aureus* in formulations with different weight ratios of ZnO nanoparticles: (**A**,**B**) images of S1–S4 samples tested against *E. coli* and their control zones using Penicillin; (**C**,**D**) images of S1–S4 samples tested against *S. aureus* and their control zones using Penicillin.



(**A**) (**B**) (**C**)

Figure 13. Biodegradability feature of the starch-based wound dressings: (**A**) 24 h; (**B**) 72 h; (**C**) Day 14.

4. Discussion

In this study, we developed and characterized a novel material for wound dressing composed of starch, polyvinyl alcohol, glycerol, citric acid, and zinc-oxide nanoparticles. To fabricate these biodegradable composites, PVA was dissolved in deionized water; starch and ZnO nanoparticles, considering the recent research interests in metal nanoparticles [44], were mixed in DI water utilizing an ultrasonic mixer; based on the existing literature [16,45–47], glycerol and citric acid were used as crosslinking agents that improved the films' flexibility and antibacterial activities. The resulting materials (S1–S4) had a rougher surface than the material (SPGC) without the ZnO nanoparticles, which promotes cell attachment and growth [48,49]. The WVTR analysis revealed that the ZnO

nanoparticles had a sufficient porosity that can cause absorption of more wound exudates and promote the wound-healing process by providing the necessary oxygen around the wound area [50]. UV-Vis spectroscopy confirmed that the material average transmittance percentage decreases after adding the ZnO nanoparticles. These starch films demonstrated advantageous UV light barrier properties as well as significant antibacterial activity against *E. coli* and *S. aureus*. Other research groups have reported the same UV–Vis spectroscopy results in similar ZnO applications over the past few years [38,39,51]. Utilizing SEM imaging, the surface morphology and structure of the wound dressings were examined in order to evaluate sponge formation when nZnO concentration was increased. The starch/PVA/nZnO composite generated by the casting method had good swelling, gel fraction, and mechanical properties to maintain a moist and antibacterial environment for wounds. The ZnO nanoparticles acted as fillers to reinforce the composite, leading to higher tensile strength and more stable and flexible wound dressings [7,17].

Moreover, the attained degradation physical characteristics of the starch/PVA/nZnO films showed that the results are the same as studied wound dressings' properties in the literature [52]. The prepared solution pH value was measured, and it was verified that the growth of bacteria could be detained within the wound area. The future work on this research topic could be directed towards monitoring human and animal wound-healing steps after wound protection with this type of protective material, the investigation of increasing the number of ZnO nanoparticles, changing the quantities of other contents of the solution to the dressing solution, and building a mathematical optimization model to enhance these wound dressings' performance.

5. Conclusions

In this study, nano-zinc-oxide particles were added to the starch-based/polyvinyl alcohol/citric acid/glycerol material (SPGC), shaped by the casting method. Then, the new solution (SPGCnZnO) water vapor transmission rate (WVTR) with the embedded ZnO nanoparticles was determined and evaluated by its values before adding the nZnO. The findings verified that the necessary WVTR around the wound bed could be provided adequately. The starch/PVA/nZnO wound dressing samples showed good dispersion and homogeneity by SEM analysis. Furthermore, the UV–Vis spectroscopy method demonstrated the UV light transmission of the starch/PVA/nZnO composite network. Before and after adding the nZnO particles, the two solutions were investigated and compared together. This research resulted in an enhanced wound dressing film with suitable characteristics such as excellent biodegradability, excellent mechanical properties, drug delivery, antibacterial properties, and a moist environment around the wound area to stimulate an optimal wound-healing procedure.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12084001/s1, Figure S1: Starch-based/nZnO wound-dressing-preparation steps.

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