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RESEARCH ARTICLE

PLA/TIO$_2$ NANOCOMPOSITES: PREPARATION, CHARACTERIZATION, ANTIBACTERIAL AND CYTOTOXICITY EVALUATION FOR FOOD PACKAGING

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Abstract. Poly (lactic acid) (PLA) is a polymer with promise for various applications, including food packaging and engineering composites. However, PLA is not appropriate for several applications due to its comparatively weak mechanical and thermal properties. The mechanical and thermal properties of PLA can be enhanced by combining it with other materials. The current study has two objectives: first, to synthesize and characterize PLA/titanium dioxide (TiO_2) nanocomposites using a solvent-casting method. The second objective of the present work was to evaluate the biomedical properties of the modified composites using antibacterial and cytotoxicity studies. The methodology part of the present study includes preparing five samples with different PLA:TiO_2 ratios and characterizing them using a field emission scanning electron microscope (FESEM), energy-dispersive X-ray spectroscopy (EDX), and dynamic mechanical analysis (DMA). The biomedical properties were evaluated using antibacterial activity against *Streptococcus pyogenes* (*S. pyogenes*) and *Salmonella*. Moreover, cytotoxicity of the prepared samples was evaluated using the MTT assay. The results show that the surface morphology of the PLA/TiO_2 nanocomposites was observed in FESEM as a homogeneous dispersion of TiO_2 nanoparticles in a PLA matrix. Moreover, particle size was measured from SEM images, and the prepared samples showed an average particle size of 27 to 63 nm. The storage modulus of the composites augmented with increasing filler loading, whilst the glass transition temperature decreased from 61.7 to 59.6 °C. The prepared composites showed a significant effect against Streptococcus pyogenes and Salmonella. Moreover, the cytotoxicity test showed higher cell viability against the HEK293 cell lines. The amalgamation of PLA and TiO_2 nanofiller has significant promise for food packaging applications.

Keywords: Biopolymer, nanofiller, ultrasonication, mechanical properties, homogeneous.

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1. INTRODUCTION

Over the past few years, biodegradable polymers have gained traction in the polymer sector because conventional polymers are primarily derived from petroleum, and their combustion produces carbon dioxide (CO2) emissions. As a consequence, this led to increased pollution and higher global temperatures. PLA has seen widespread use across a variety of applications, including food packaging, biomedical products, and consumer goods. Researchers worldwide are increasingly interested in developing bio-based polymeric materials to comply with current environmental policies [1].

PLA is an organic polymer that comes from one chemical substance found in living things: lactic acid. Because lactic acid is a precursor in multiple metabolic pathways and is produced by bacteria, plants, and mammals, it is one of the most significant chemicals in our bodies. As a bio-based, biodegradable polyester, PLA is made from monomers derived primarily from renewable natural resources such as sugar, corn, potatoes, and beets. Furthermore, the waste products of its products can degrade completely into CO_2 and H2O under appropriate environmental conditions [2,3], thereby reducing environmental pollution in certain applications. The qualities of PLA, such as excellent mechanical capabilities, renewability, biodegradability, competitive structure, good transparency, thermal plasticity, and low toxicity [4], offer it the potential for application in industries such as medicine, food packaging, agriculture, textiles, and other fields in a variety of diverse forms. Despite this, the material has a low thermal stability due to its high rigidity and brittleness. [5], and relatively low crystallization rate [6] limit their large-scale applications. Modification of PLA is necessary to widen its application; thus, many research efforts have been directed toward overcoming those limitations, such as the addition of plasticizers [7], nanoparticles [8], natural fibers [9], and polymer blending [10].

Recently, nano-inorganic materials such as TiO_2 [6], ZnO [7], Ag [8], and SiO_2 [9] have become widely used in many fields. The incorporation of nanostructured fillers, which can act as a mechanical reinforcement of the structure of different polymers, because they possess good stability under different temperatures and pressure conditions, and also offer good antibacterial and ultraviolet (UV) properties. Nanostructured fillers are considered safe for human beings in comparison to organic substances [10]. Although PLA can form a strong interface with various solid surfaces, TiO_2 is an important chemical and environmental material. Nano-TiO_2 particles possess many excellent properties, such as photocatalytic activity, photo stability, non-toxicity, biocompatibility, intense UV absorption, and antimicrobial activity. TiO_2 shows better interfacial adhesion to PLA due to chemical bonds between TiO_2 and lactic acid [11].

Extensive research on biodegradable polymers and functional nanomaterials has been prompted by the worldwide demand for high-performance, environmentally sustainable food packaging materials [11]. Due to its superior transparency, biocompatibility, and mechanical properties, PLA, a renewable and compostable polyester derived from agricultural resources, has become a viable option. However, intrinsic disadvantages such as low heat stability, brittleness, and weak antibacterial properties frequently limit its practical application in food packaging. The use of inorganic nanoparticles, particularly TiO_2, has garnered increasing attention as a means to circumvent these restrictions. TiO_2 nanoparticles are effective enhancers for polymer-based packaging materials due to their unique physicochemical properties, including a high surface area, strong UV-blocking capacity, photocatalytic activity, and potent antibacterial effects.

Researchers are interested in simultaneously enhancing mechanical strength, barrier performance, thermal stability, and microbiological resistance by creating PLA/TiO_2 nanocomposites [6-10]. Furthermore, assessing the cytotoxicity and antibacterial behaviour of PLA/TiO_2 systems is crucial to ensuring the safe practical use of these systems, given growing concerns regarding the potential toxicity of nanomaterials in food-contact applications. Thus, this work contributes to the development of next-generation biodegradable food packaging materials with enhanced safety and functionality by producing and thoroughly characterising PLA/TiO_2 nanocomposites and evaluating their cytotoxicity and antibacterial efficiency.This study aims to develop and characterise PLA/ TiO_2 nanocomposites and to evaluate their antibacterial activity and cytotoxicity, thereby determining their suitability and safety for advanced food packaging applications.

2. MATERIALS AND METHODS

2.1 Materials

TiO_2 nanoparticles with high purity 99.5% and particle size below 100 nm were purchased from Sigma–Aldrich (USA), while the Poly (lactic acid) was purchased from NatureWorks.

2.2 The Preparation Process of PLA /TiO_2 Nano Composites

The solvent-casting method was used to prepare PLA/TiO_2 nanocomposites with varying filler loadings. The method was used with Tetrahydrofuran (THF), as shown in Table 1.

Table 1: Material designation and Nano composition of PLA / TiO_2

No	PLA (wt%)	TiO_2(wt.%)	Material designation
1	99.5	0.5	$PTiO_2$-0.5
2	98.0	2.0	$PTiO_2$-2.0
3	97.5	3.5	$PTiO_2$-3.5
4	95.0	5.0	$PTiO_2$-5.0
5	92.5	7.5	$PTiO_2$-7.5

PLA pellets were dried at 60 °C for 24 hours to remove moisture, then dissolved in THF with a magnetic stirrer for 6 hours (5 g PLA in 100 mL THF). Precisely weighed TiO_2 nanoparticles were dispersed in THF for 1 hour with a mechanical stirrer, followed by 30 minutes with an ultrasonicator. The PLA solution was slowly added during 6 hours of agitation to ensure good distribution and reduce agglomeration. Samples were cast onto petri dishes and left to evaporate the solvent for 24 hours. To eliminate any remaining solvent, the thin PLA nanocomposite coating was baked at 45°C for 24 hours. Finally, samples were pressed in a heated compression machine to ensure uniformity and prevent bubbles.

2.3 Nanocomposites Characterization

FESEM was commonly used to observe the surface morphology and shape of PLA and a PLA nanocomposite (sputter-coated with platinum). This investigation used the Hitachi SU8020 FESEM equipment. The FESEM was also an EDX (Horiba EMAX) device used to determine the elemental composition of the samples, which were irradiated with an electron beam. EDX analysis required 60 seconds per point for the samples. Moreover, the Dynamic mechanical analysis (DMA) test was conducted using the TA (DMA Q800) instrument, operating in three-point bending mode at an oscillation frequency of 1 Hz with a regulated amplitude. Experimental parameters include a heating rate of 50 °C/min and an operating temperature range of 20 to 160 °C. The samples were in the rectangular specimens of (19 × 8.5 × 0.52) used for the analysis.

2.4 Antibacterial activity and Cytotoxicity of PLA/TiO_2 Nanocomposites

The synthesized PLA/TiO_2 nanocomposites were assessed for their efficacy against *Streptococcus pyogenes* and *Salmonella* using the disc diffusion technique. Natural agar (NA) for the cultivation of bacterial strains. After the NA medium solidified, wells with a 5 mm diameter were created using a cork borer. The bacterial culture was uniformly spread on each agar plate. In each well, $PTiO_2$-0.5, $PTiO_2$-2, $PTiO_2$-3.5, $PTiO_22$-5.0, and $PTiO_2$-7.5 were introduced, and the plates were incubated at 37 °C for 24 hours. After incubation, the inhibition zone was quantified. Each experiment was conducted in triplicate.

The MTT test assessed the cytotoxicity of $PTiO_2$-0.5, $PTiO_2$-2, $PTiO_2$-3.5, $PTiO_22$-5.0, and $PTiO_2$-7.5 on HEK293 cells. Cell viability was evaluated by quantifying the conversion of the yellow tetrazolium salt MTT to the blue formazan product via mitochondrial dehydrogenase activity. The cells were inoculated onto a 96-well plate at a consistent density of 1×10^4 cells per well and allowed to adhere and grow overnight in a 5% CO_2 incubator. Following a 24-hour incubation period, the monolayer was rinsed with fresh media, and the adherent cells were incubated with varying concentrations of each sample (5, 10, 15, 30, and 50 µg/mL) for an additional 24 hours. To assess cell viability, 10 µL of MTT dissolved in PBS was introduced to each well. The plates were then incubated in the dark at 37 °C in a humidified environment containing 5% CO_2 for 4 hours. The supernatant was combined with 100 µL of DMSO to solubilize the formazan product. The absorbance of the plates was measured at 550 nm. A control medium without bimetallic nanoparticles at the designated concentrations was included.

3. RESULTS AND DISCUSSION

3.1 *FESEM of PLA/TiO_2 Nanocomposites*

Figure 1 presents FESEM and EDX micrographs of PLA nano-composites with varying concentrations of nano-TiO_2 at 0.5, 2.0, 3.5, 5.0, and 7.5. The nanoparticles are disseminated inside the material; nevertheless, an increase in the addition of nano-TiO_2 to the nano-composites leads to some aggregation. The aggregation propensity may be elucidated by its association with agglomeration events arising from the contact between the Ti-OH groups on particle surfaces [12], no surface treatment was conducted on the oxide nanoparticles, and the fabrication of nano-composites included casting the solution and directly dispersing TiO_2 nanoparticles in PLA solution. The existence of TiO_2 nanoparticles was confirmed by elemental analysis of the PLA nanocomposite using EDX.

The interfacial interaction between PLA chains and TiO_2 particles remained restricted since the production procedure involved the direct dispersion of TiO_2 nanoparticles into the PLA solution without the application of coupling agents, surfactants, or other surface-modifying techniques. As a result, at higher TiO_2 concentrations, van der Waals forces and hydrogen bonds between nanoparticles predominate, thereby promoting the formation of larger clusters [13]. As already noted in comparable polymer–oxide nanocomposites [14], such clustering may affect the homogeneity of the composite's microstructure as well as its mechanical, thermal, and optical properties.

Figure 2(a) shows the EDX graph of the PLA and shows the presence of carbon (C), Oxygen (O), and chloride (Cl), while Figure 2(b) to (f) showed the presence of Ti as metallic at 4.5 and 5 kev. As mentioned in the previous studies, the peak of the metallic Ti ion is located at 4.5 and 5 kev [15]. The dispersion and existence of TiO_2 nanoparticles in the PLA matrix are verified by the EDX elemental analysis. The morphological findings from SEM are supported by the increase in Ti signal intensity with increased nanoparticle loading, confirming that TiO_2 content directly influences dispersion quality and the onset of agglomeration. Overall, these results show that while lower concentrations of TiO_2 can be successfully dispersed within PLA, higher loadings require more effective dispersion techniques to prevent aggregation and preserve advantageous composite properties, such as surface modification, ultrasonication optimization, or the use of compatibilizers [13,14].

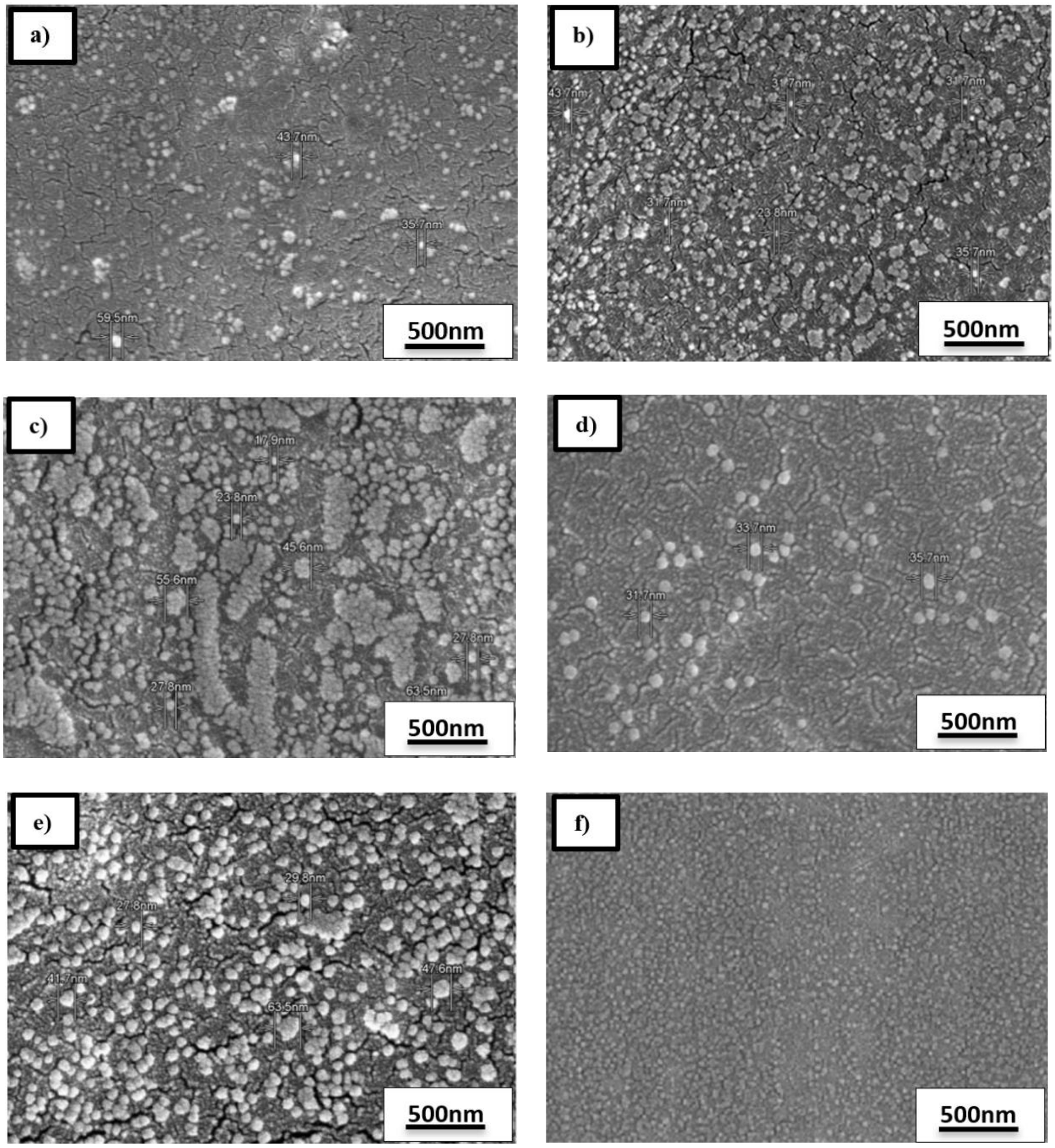
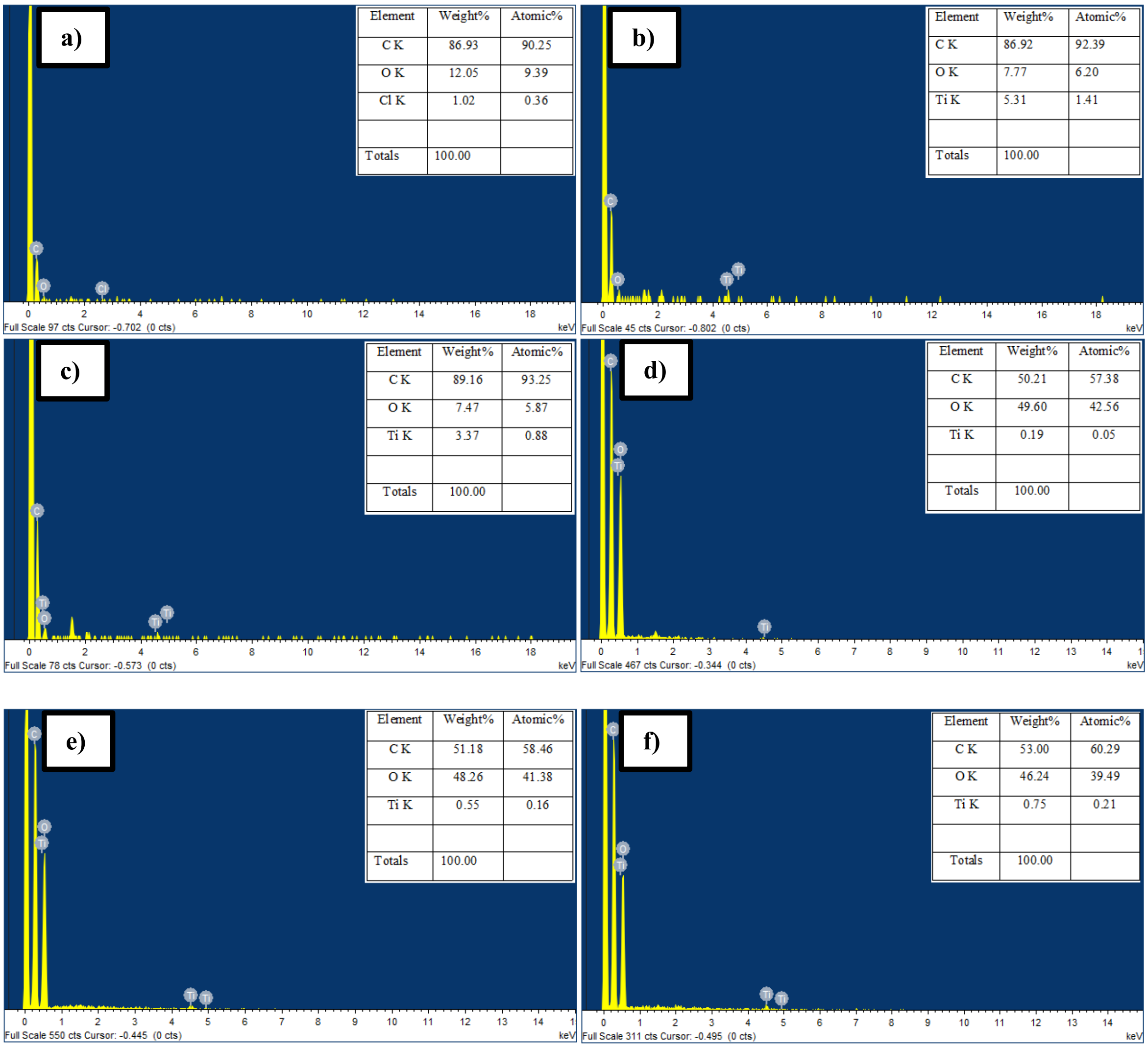


Figure 1: FESEM micrographs of PLA/ nanocomposites; (a) PLA/0.5%TiO_2, (b) PLA/2%TiO_2, (c) PLA/3.5%TiO_2, (d) PLA/5%TiO_2 (e) PLA/7.5%TiO_2 and (f) PLA



a)

Element	Weight%	Atomic%
C K	86.93	90.25
O K	12.05	9.39
Cl K	1.02	0.36
Totals	100.00	

b)

Element	Weight%	Atomic%
C K	86.92	92.39
O K	7.77	6.20
Ti K	5.31	1.41
Totals	100.00	

c)

Element	Weight%	Atomic%
C K	89.16	93.25
O K	7.47	5.87
Ti K	3.37	0.88
Totals	100.00	

d)

Element	Weight%	Atomic%
C K	50.21	57.38
O K	49.60	42.56
Ti K	0.19	0.05
Totals	100.00	

e)

Element	Weight%	Atomic%
C K	51.18	58.46
O K	48.26	41.38
Ti K	0.55	0.16
Totals	100.00	

f)

Element	Weight%	Atomic%
C K	53.00	60.29
O K	46.24	39.49
Ti K	0.75	0.21
Totals	100.00	

Figure 2: EDX spectrum of PLA/ nanocomposites; (a) PLA, (b) PLA/0.5%TiO_2, (c) PLA/2%TiO_2, (d) PLA/3.5%TiO_2, (e) PLA/5%TiO_2 and (f) PLA/7.5%TiO_2,

3.2 DMA *PLA/TiO_2 Nanocomposites*

Figure 3 shows the storage modulus (E′) variation with temperature of pure PLA and PLA/TiO_2 nanocomposites. The storage modulus curve shown in fig.1 is divided into three characteristic regions. At low temperatures 30 to 50 °C, the PLA composites are in the glassy state and exhibit brittle, rigid behaviour. In the glass transition region 61.60 to 59.63, all samples exhibited typical behaviour of a semi-crystalline polymer, with a large drop in the storage modulus corresponding to their glass transition temperature (Tg), where peaks in the loss modulus and damping factor appear. The composite's behaviour changes from brittle and rigid to soft and ductile. The third region is the rubbery state, where stiffness is very low and the material retains its softness and elastic behaviour. The incorporation of TiO_2 enhanced the storage modulus of composites relative to pure PLA in both the glassy and rubbery states. This indicates increased stiffness in both domains, which aligns with the nanofiller's reinforcing effect. The glass transition temperature is the range at which a thermosetting polymer transitions from a stiff, glassy state to a more flexible, elastic one. The storage modulus began to rise at around 100 °C, attributable to the cold crystallisation of PLA.

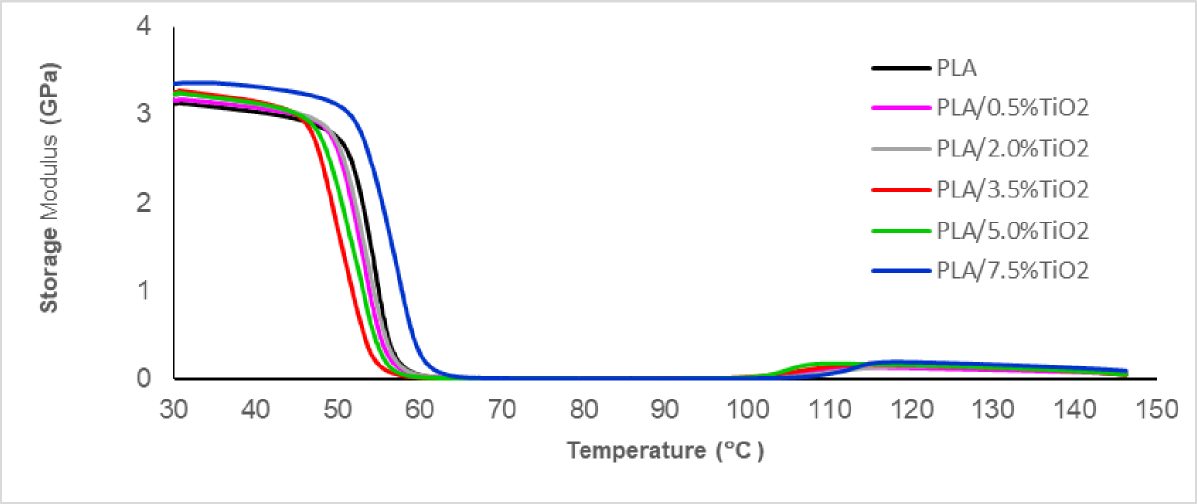


Figure 3: Storage modulus different with a temperature of pure PLA and PLA/TiO$_2$ nanocomposites

The loss modulus (E″) reflects the viscous behavior of materials and indicates their propensity to lose applied energy. Figure 4 presents the E″ curves of PLA and the nanocomposites. The loss modulus for all tested sample ratios increased with increasing nanoparticle concentration over the entire temperature range analyzed. All loss modulus curves show a maximum in dissipated mechanical energy, which diminishes at elevated temperatures due to the increased mobility of the polymer chains. Additionally, the incorporation of TiO$_2$ nanofiller reduces the viscosity of the nanocomposite compared to pure PLA [16]. Both above and below the glass transition temperature (the apex of the E″ curve), the E″ of pure PLA exceeded that of the PLA/nanocomposites.

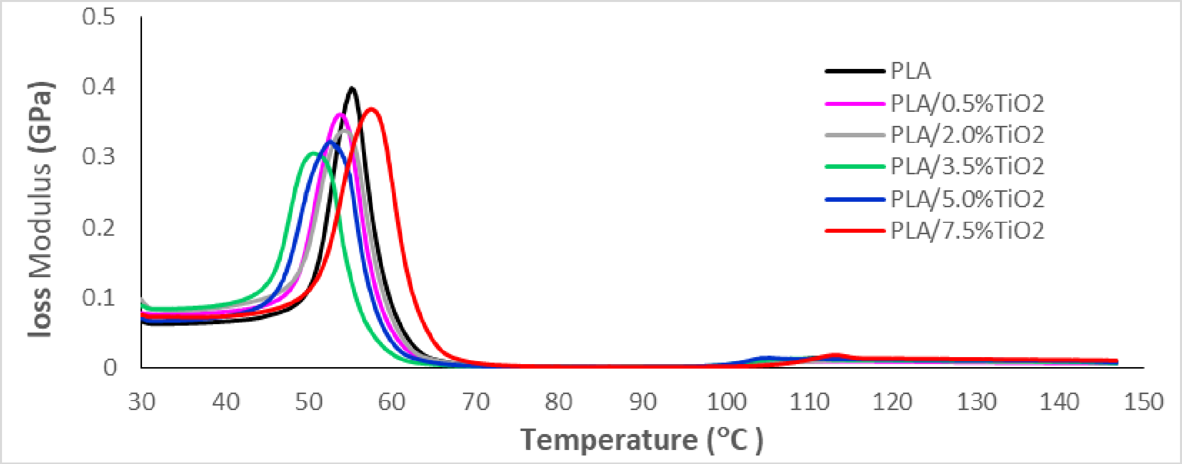


Figure 4: Loss modulus different with a temperature of pure PLA and PLA/TiO$_2$ nanocomposites

Figure 5 shows the fluctuation of tan δ with temperature for pure PLA and PLA/TiO$_2$ nanocomposites. The peak temperature of Tan δ signifies the glass transition temperature (Tg) of materials. The incorporation of TiO$_2$ resulted in a slight shift of the tan δ peaks to lower temperatures, from 61.6 to 59.63 °C [17,18]. PLA/nanocomposites exhibit a reduction in the tan δ peak height compared to plain PLA. The mtan δ peak's magnitude correlates with the amorphous area's chain mobility in the polymer nanocomposites. The determined Tg, E′, E″, and tan δ temperatures for the blank and PLA nanocomposite are shown in Table 2. The thermo-mechanical properties of PLA nanocomposites were analysed using DMA and the results are show in Figures 1, 2 and 3 and the data are summarized in Table 2.

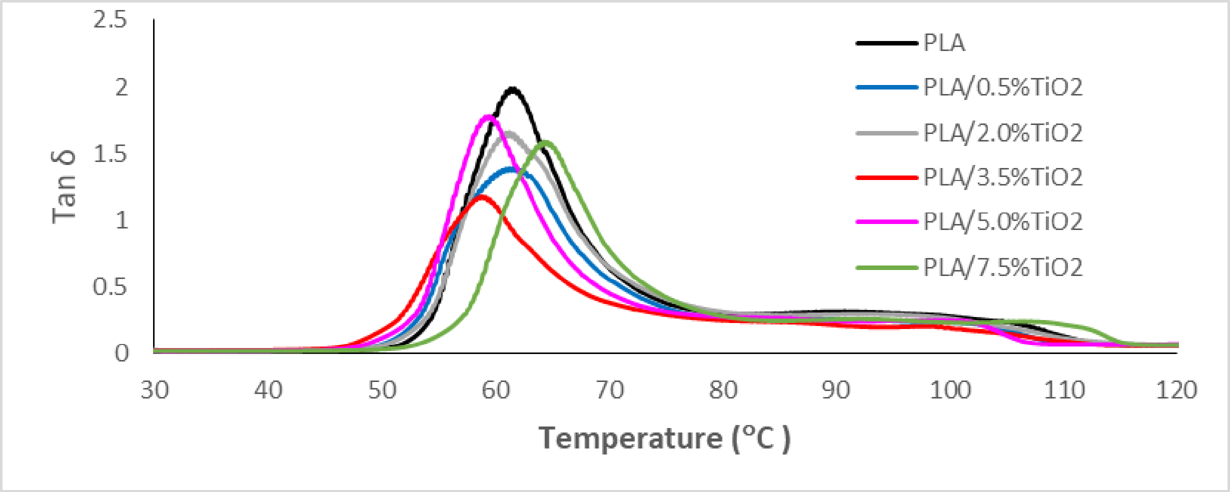


Figure 5: Tan δ different with a temperature of pure PLA and PLA/TiO_2 nanocomposites

Table 2: Thermomechanical parameters of PLA and PLA/TiO_2 nanocomposites

Samples	**E′ (GPa)**	**E″ (GPa)**	**T_g (°C)**	**tan δ**
PLA	3.131	0.071	61.60	1.971
$PTiO_2$-0.5	3.216	0.081	60.69	1.38
$PTiO_2$-2.0	3.190	0.115	61.22	1.643
$PTiO_2$-3.5	3.264	0.092	59.63	1.172
$PTiO_2$-5.0	3.234	0.077	60.95	1.764
$PTiO_2$-7.5	3.364	0.074	60.82	1.58

3.3 Antibacterial of *PLA/TiO_2 Nanocomposites*

The prepared materials, including PLA, PLA/0.5%TiO_2, PLA/2%TiO_2, PLA/3.5%TiO_2, PLA/5%TiO_2, and PLA/7.5%TiO_2, were cured and evaluated for their antibacterial activity against Streptococcus *pyogenes* (*S. pyogenes*) and *Salmonella* using the disc diffusion technique. As shown in Table 3, the PLA didn't show any effect against either of the bacteria. These results agree with previous studies, as reported by Suganthi et al. [19], who found that PLA alone did not show any effect against bacteria. While the prepared samples with TiO_2 showed significant effects against both strains of the bacteria, as observed in the figure, the inhibition zone of the bacteria in the PLA/5%TiO_2 was the highest one, which showed 16 mm and 15 mm against *S. pyogenes* and *Salmonella*, respectively, compared with PLA/3.5%TiO_2, which showed 12mm and 11mm against them. The present study showed that the presence of TiO_2 imparted significant properties to the prepared composites.

Table 3: The antibacterial effect of PLA, PLA/0.5%TiO_2, PLA/2%TiO_2, PLA/3.5%TiO_2, PLA/5%TiO_2, and PLA/7.5%TiO_2 against *S. pyogenes* and *Salmonella*

No	**Prepared Composites**	***S. pyogenes***	***Salmonella***
1	PLA	0 mm	0 mm
2	PLA/0.5%TiO_2	14mm	11mm
3	PLA/2%TiO_2	13mm	10mm
4	PLA/3.5%TiO_2	12mm	11mm
5	PLA/5%TiO_2	16mm	15mm
6	PLA/7.5%TiO_2	14mm	12mm

As observed in Figure 6, the prepared composites exhibit significant inhibition of *S. pyogenes* and *Salmonella*. The study by Xing et al. [20] reported the fabrication of a polyethylene (PE)/TiO_2 composite. It investigated their antibacterial activity against *Escherichia coli (E. coli*) and *Staphylococcus aureus (S. aureus*), with inhibition zones of 16 and 20 mm, respectively. TiO_2 enhanced the prepared composites' suitability for biomedical applications, consistent with the present study's outcome. Moreover, based on the antibacterial test results, the present study aimed to prepare modified PLA composites with promising antibacterial activity, making them excellent preventers for use in food packaging.

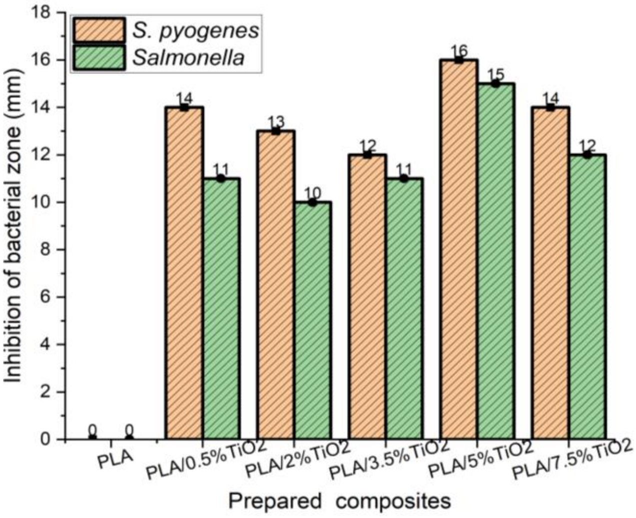


Figure 6: The inhibition zone of the bacteria of the prepared composites of PLA

3.4 Cytotoxicity Test of PLA/TiO_2 Nanocomposites

The MTT method was used to evaluate the cytotoxicity of the prepared PLA composites: PLA, PLA/0.5% TiO_2, PLA/2% TiO_2, PLA/3.5% TiO_2, PLA/5% TiO_2, and PLA/7.5% TiO_2. Five concentrations of each prepared composite were chosen: 5, 10, 15, 30, and 50 µg/mL. Table 4 shows the details of the cultured prepared samples with the HEK293 cell lines.

Table 4: The MTT assay of PLA prepared composite

Conc µg/mL	**Control**	**1**	**2**	**3**	**4**	**5**	**6**
5	0.356	0.341	0.344	0.361	0.337	0.362	0.355
10	0.307	0.302	0.298	0.284	0.299	0.279	0.31
15	0.219	0.221	0.204	0.195	0.211	0.212	0.215
30	0.301	0.297	0.289	0.282	0.286	0.294	0.288
50	0.274	0.282	0.251	0.273	0.267	0.264	0.269
				Cell viability			
Conc µg/mL	**Control**	**1**	**2**	**3**	**4**	**5**	**6**
5	95.78	96.62	96.62	101.40	94.66	101.68	99.71
10	98.37	97.06	97.06	92.50	97.39	90.87	100.97
15	100.9	93.15	93.15	89.04	96.34	96.80	98.17
30	98.67	96.0	96.01	93.68	95.01	97.67	95.68
50	102.92	91.60	91.60	99.63	97.44	96.35	98.17
Average	99.33	94.89	96.49	95.25	96.17	96.67	98.54

(**Conc**; Concentrations, **1**; PLA, **2**; PLA/0.5%TiO_2, **3**; PLA/2%TiO_2, **4**; PLA/3.5%TiO_2, **5**; PLA/5%TiO_2, **6**; PLA/7.5%TiO_2)

Figure 7 shows the cell viability after culturing the prepared composites for 24h; the outcome shows all concentrations were non-toxic with cell viability of 94%; this outcome gave the evidence to use the prepared PLA composites safely in food packaging and biomedical applications.

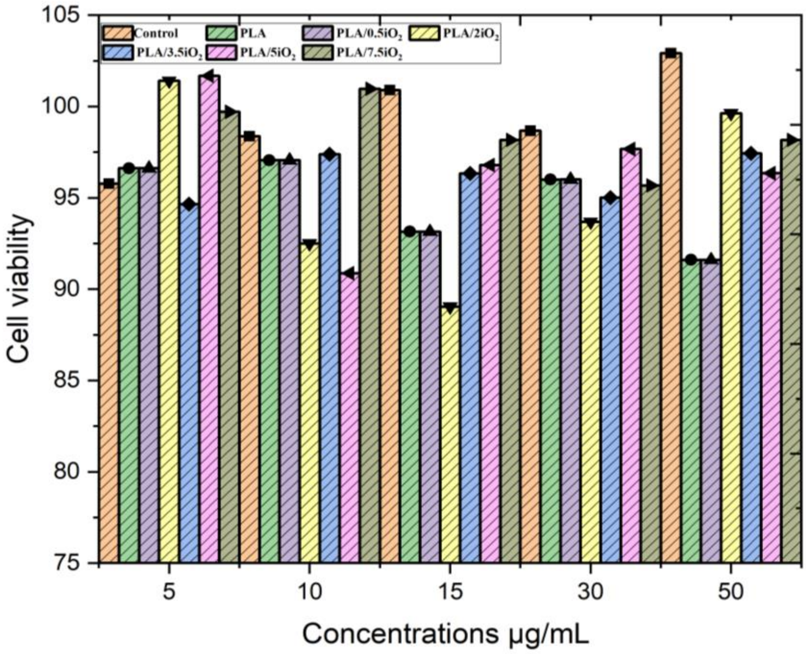


Figure 7: The cell viability of the prepared PLA composites with TiO_2

4. CONCLUSIONS

The solvent casting process was successfully employed in this study to produce PLA/TiO_2 nanocomposites with varying nanoparticle loadings (0.5–7.5 wt%), and their morphological and dynamic mechanical properties were systematically evaluated. The inclusion of TiO_2 has a significant impact on the viscoelastic properties of PLA, as determined by dynamic mechanical analysis. In comparison to clean PLA, the storage modulus of the nanocomposites increased, indicating higher stiffness and better load-bearing capacity over the temperature range under investigation. On the other hand, as the TiO_2 content increased, the loss modulus and tan δ values consistently decreased, indicating a decrease in molecular mobility and damping properties within the composite matrix. In particular, the tan δ peak dropped from 1.97 for pure PLA to 1.171 at a 7.5 weight per cent TiO_2, demonstrating the nanoparticles' limited segmental mobility. As the TiO_2 loading increased, the glass transition temperature (Tg) also decreased slightly, from 61.6 °C to 59.63 °C, which was attributed to the increased free volume and chain mobility of the nanoparticles. Overall, the findings indicate that the addition of TiO_2 enhances PLA's mechanical stability while maintaining its favourable damping and thermal properties. The produced PLA/TiO_2 nanocomposites also exhibited encouraging antibacterial activity with minimal cytotoxic effects, highlighting their potential relevance for biomedical applications and other domains that require mechanically reinforced, biocompatible materials.

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Author Contributions

All authors contributed toward data analysis, drafting, and critically revising the paper, and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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