Preparation of Cellulose Acetate Nanocomposite Films Based on TiO$_2$-ZnO Nanoparticles Modification as Food Packaging Applications

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Abstract
To develop bio-packaging materials, nanocomposite films of cellulose acetate reinforced with titanium dioxide and zinc oxide nanoparticles were prepared, by the casting method at different weight ratios of ZnO nanoparticles (1.5, 2, and 2.5) wt% and a constant weight ratio of 2 wt% TiO$_2$. ZnO and TiO$_2$ nanoparticles were tested using scanning electron microscopy (SEM). The mechanical properties (tensile strength and elongation) were improved at a fixed level of Cellulose Acetate+ 2% TiO$_2$+1.5 wt% ZnO loading. Beyond that level of loading, they decreased. The tensile strength was decreased due to some degrees of agglomeration of filler particles above a critical content. Fourier-Transform Infrared Spectroscopy (FTIR) was conducted to reveal the microstructures and chemical composition of as-prepared composite films. The wettability of the films was also determined by the sessile drop method. An increase in contact angle was also observed by the addition of ZnO content from 70.6° to 77.1° compared to pure Cellulose Acetate, which indicated a value of 61.3°. Antibacterial activity against Escherichia coli and Staphylococcus aureus was enhanced after incorporation of ZnO-TiO$_2$ compared with pure CA. The enhanced wettability and antibacterial activity of the prepared films suggest that they could be used for packaging applications.

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1. Introduction
Food packaging and contact surfaces with food have been a cause of worry in the food industry since they do not provide enough protection or maintain the freshness of products. However, the possibility of extending the shelf life of foods via microbial resistance as well as lowering the growth rate of bacteria has been a focus of academic and corporate research. Packaging technology offers limitless possibilities for food preservation, and Cellulose Acetate (CA), which is made from naturally occurring cellulose materials such as wood, cotton, and rice husk, is a green product that has been nominated for use in the creation of food packaging films. It is favored for its superior appearance, biodegradability, greater durability, and nontoxicity [1-3]. It is applied in several ways, including filters, medication delivery, and medical implants [4-7]. Due to their edible, renewable, and...
biodegradable qualities, biopolymer-based films have been often used in packaging [8-12]. To increase the functionality of packaging, nanoparticles such as zinc oxide and titanium dioxide have been used to create composite functional films [13, 14]. Because of their stability, ability to suppress bacterial growth, and ability to prevent the formation of new cell structures, TiO$_2$ nanoparticles have gained substantial attention [15]. These nanoparticles have improved mechanical capabilities, which are stable under hard circumstances, and are antibacterial owing to the active oxygen species formed by metal oxide particles [16]. The US Food and Drug Administration (USFDA) has approved the use of TiO$_2$ and ZnO nanoparticles as food additives and food contact chemicals after undertaking a safety investigation [17, 18]. The insertion of nanoparticles such as zinc oxide (ZnO) and titanium dioxide (TiO$_2$) nanostructures into cellulose acetate has been suggested to remove dithiotrethiol (DTT) from water and to be utilized as a covering for wood preservation [19-21]. Nanocomposites of cellulose acetate based on Au/ZnO showed a greater water contact angle and increased photocatalytic activity than pure cellulose acetate [22, 23]. Additionally, it has been demonstrated that black grapefruits with ZnO-incorporated cellulose acetate phthalate and chitosan have a longer shelf life [24]. The current study focuses on the synthesis and application of cellulose acetate thick films that combine ZnO-TiO$_2$ nanoparticles. It is different from previous studies in that it incorporates different materials and conducts various examinations for food packaging applications. The physical and chemical properties of the ZnO-TiO$_2$ nanocomposite films, as well as their antibacterial activity and contact angle, are investigated.

2. Experimental Procedure

2.1. Materials

CA (CDH India, acetyl content 29-45%; Maximum limit of impurities; 0.1%; free acid (as acetic acid); 5.0%; loss on drying at 105°C sulphated ash; 0.1%) and Acetone AR/ACS (2-Propanone, Dimethylketone) (M.W.:58.08) from (CDH India). Titanium oxide nanoparticles/nanopowder (TiO$_2$, Rutile, 99.5%), particle size (20-40) nm, Zinc Oxide nanoparticles (99.8%), particle size (20-50) was purchased from (Skyspring, USA).

2.2. Methodology

2.2.1. Preparation of primary polymer solutions

The main solution of CA was made using the casting technique by dissolving 7 g of CA in 100 ml of acetone. After 8 hours of stirring with a magnetic stirrer, the solution was cast on a glass petri dish, and a final film was achieved after 48 hours.

2.2.2 Preparation of ZnO/TiO$_2$/CA composite polymer solution

ZnO and TiO$_2$ nanoparticles with C$_3$H$_6$O Solution were added to CA Powder at a different weight ratio of ZnO nanoparticle (1.5, 2, and 2.5 wt.%) and a constant weight ratio of 2%TiO$_2$ of the composite film. The mixture was stirred until getting the homogeneous solutions; Then the solution was cast in a glass Petri dish and left to dry for 48 hrs. The preparation process was shown in Figure 1.

2.3. Characterization

The morphology of the powder was examined with (FESEM Zeiss Sigma 300-HV Germany). The mechanical characteristics of the cellulose acetate and the composite’s thick films were measured by tensile tests using Laryee Universal Testing Machine (UTM) UE3450 from (Laryee Technology Co., Ltd. China). The films were cut to 120 mm × 10 mm, and the thickness was measured by a digital micrometre with ±1 μm accuracy. All the tests were performed at room temperature (around 27°C) and a crosshead speed of 5 mm/min. was used during the measurements of the cellulose-based films, and a stress-strain curve was recorded. Young’s modulus, elongation at the breakpoint, and ultimate tensile strength were determined using the equation below [25]:
Young modulus (Mpa) = Stress (Mpa)/ Strain  \hspace{1cm} (1)

\text{Stress (Mpa) = Force (N)/ Area (mm}^2\text{)} \hspace{1cm} (2)

\text{Strain = Elongation (%) = } \frac{\Delta L}{L_0}\times 100\% \hspace{1cm} (3)

\Delta L (mm) = L_1 - L_0 (mm) \hspace{1cm} (4)

Where \(\Delta L\): is the amount of the change that occurs in length, \(L_1\): is the length after the deformation, \(L_0\) is the original length.

Ultimite tensile strength (Mpa) = \(\frac{P_{max} (N)}{A (mm^2)}\) \hspace{1cm} (5)

Where \(P_{max}\): Maximum tensile load a sample can withstand during the test, \(A\): the Area. Fourier transformed infrared (FTIR) spectra were obtained by (SHIMADZU-8400S FTIR spectrometer, Japan). The spectra were obtained in the wavenumber range of 400-4000 cm\(^{-1}\), 4 cm\(^{-1}\) resolutions. The samples were prepared as tablets by mixing with KBr powder.

![Figure 1: A schematic diagram of the preparation process for the films, on the right, the device used to prepare the compound (CA) and the final solution of the films (CA/TiO\(_2/\)ZnO).](image)

The optical system used to measure contact angle was from (Holmarc Opto-Mechatronics P Ltd. India), with an automated dispenser and software for static and dynamic contact angle measurement. A drop of water is placed on the film surface and it will spread on the surface based on the interactions between the solid surface and the water. Water contact angle will be measured to indicate the wettability of the surface. The antibacterial activity of the CA and CA/TiO\(_2/\)ZnO was tested against human pathogens Escherichia coli and Staphylococcus aureus using the following protocol. The bacteria were captured from their stock cultures using a sterile wire loop. To assess how CA and CA/TiO\(_2/\)ZnO have an impact on the bacteria's growth curve, the bacterial strains were grown at 37\(^\circ\) C on M-H agar plates with inoculations of 50 mL of nutrient broth. The bacterial growth grew until the nutritional broth attained an optical density (OD) of 0.1 at 600 nm, which corresponds to a bacterial concentration...
of 108 CFU/mL. The bacterial cultures (1 mL) were then added to the nutritional broth along with CA and CA/TiO$_2$/ZnO, and incubated at 37°C for 12 hours with slight agitation. The OD was measured with a spectrophotometer to determine bacterial growth. An unpaired t-test was used to statistically assess the obtained data. The results were provided as the mean ± SD of triplicate measurements.

3. Results and Discussion
3.1. SEM Analysis
SEM micrographs of TiO$_2$ and ZnO powders are shown in Figure 2. As demonstrated, TiO$_2$ and ZnO nanopowders have extremely small particle sizes with a narrow particle size distribution of around 20-40nm and 20-50nm, respectively. Due to the increased surface area of fine particles, a more complex dispersion approach will be necessary to avoid agglomeration and to provide a more homogeneous particle size distribution within the CA matrix.

![Figure 2: SEM images of (a) TiO$_2$ nanoparticle, (b) ZnO nanoparticle.](image-url)

4.2. Tensile Test
As shown in Figure 3, the stress-strain curves for ZnO-TiO$_2$/CA composite films were obtained using varied weight ratios of ZnO nanoparticles (1.5, 2, and 2.5%) in addition to a constant weight ratio of 2 wt% TiO$_2$ in the composite film.

Improved mechanical characteristics were seen when inorganic fillers were distributed evenly throughout the (CA) polymer matrix, with up to 1.5 weight percent zinc oxide and 2 weight percent titanium dioxide being used. Significant parameters were taken from Figure 3 and displayed in Figures 4, and 5, demonstrating their variation with ZnO concentrations [26-28].
**Figure 3:** The stress-strain curve of ZnO/TiO$_2$ with Cellulose acetate.

**Figure 4:** Variation of the ultimate tensile strength and elongation of CA/TiO$_2$ films with different ZnO content.
The intermolecular force chain, stiffness, and molecular symmetry of polymer systems all influence the mechanical characteristics of polymer systems [29]. Polymers with a high degree of crystallinity, crosslinking, or rigid chains have high strength or limited extendibility, resulting in a high yield modulus, high stress at peak value, and a low elongation value. Because of electrostatic interactions between the ester atoms of one chain and the hydroxyl group of another, CA is a stiff and strong material that shows dipole-dipole attraction between the two chains. The interaction of dipole-dipole attraction, which achieves its greatest magnitude, was shown to relate to an improvement in the mechanical properties of materials. A significant increase in the filler's ability to cling to the matrix was associated with a decrease in the amount of sliding between the composite layers when stress was applied to the composites [30, 31]. The tensile strength is reduced due to some degrees of agglomeration of filler particles above the critical content and an increase in inhomogeneity [27, 28]. The lack of interfacial adhesion between the polymer and the fillers was responsible for the decrease in tensile strength [31]. At a fixed level of 1.5%ZnO-2% TiO$_2$/CA loading; both tensile strength and elongation were improved as shown in Figure 4, beyond that level of loading the values of tensile strength and elongation decreased. The general behaviour of the Youngs modulus was found to be dependent on the elongation and ultimate strength according to the filler contents and the homogeneity of particle distribution within the CA matrix.

4.3. FTIR Spectroscopy
Figure 6 showed FTIR spectra of pure CA film and CA films containing different weight ratios of ZnO nanoparticle (1.5, 2, and 2.5) wt% and a constant weight ratio of 2 wt%TiO$_2$. The film spectrum is characterized by the presence of bands at 1741 cm$^{-1}$ (steric carbonyl stretching), 3478 cm$^{-1}$ (cellulose OH stretching), and 2936 cm$^{-1}$ (CH stretching) [32]. The bands of 2936 cm$^{-1}$ (CH stretching), 3478 cm$^{-1}$ (OH stretching), 1741 cm$^{-1}$ (steric carbonyl stretching), 1232 cm$^{-1}$, and 1045 cm$^{-1}$ (C-O stretching), all increased when ZnO-TiO$_2$ nanoparticle were added. Because TiO$_2$ is an oxide with O-Ti-O bonding in its chemical structure, inserting it into cellulose acetate may have increased the interactions between the two compounds at the region of the band that represented these properties. TiO$_2$ bonding is represented by peaks at 600 cm$^{-1}$ and 750 cm$^{-1}$ [33, 34]. Carbonian and hydroxyl groups have peaks cantered at 1750 cm$^{-1}$ and 3478 cm$^{-1}$, respectively [32]. The vibration frequency of O-Zn-O bond groups is indicated by the peak at 400-600 cm$^{-1}$ [24].
4.4. Water Contact Angle
Wettability testing measures how many water molecules a sample can absorb in a certain period. The wettability character of a surface is described as whether or not the sample is hydrophilic or hydrophobic, in general. Figure 7 depicts the water contact angles for CA and ZnO-TiO$_2$/CA at varied weight ratios of ZnO nanoparticle (1.5, 2, and 2.5) wt% and a constant weight ratio of 2 wt% TiO$_2$. Its hydrophilic nature resulted in a contact angle of 61.3° for the CA film, which was the lowest of all the films tested. Low surface wettability leads to a high contact angle and vice versa [35], hence the CA film had the lowest surface wettability of all the films tested. When compared to CA film, all-composite films had greater contact angles of 70.6°, 72.9°, and 77.1°, respectively. The contact angle of the produced films increased in the direction of hydrophobicity as the amount of nano ZnO present in them increased [22].
Figure 7: water contact angle for CA film and nanocomposite thick films with various ZnO content. The inset pictures show the images for the measurement of surface contact angles.

4.5 Antibacterial Activity
The Optical Density (OD) of Escherichia coli and Staphylococcus aureus suspensions at 600 nm treated with different films in test tubes for 12 hours was measured, which is a commonly used method for determining the growth of bacteria for the assessment of antibacterial activities [36, 37]. In general, the lower the optical density of bacteria suspension at 600 nm after a specific period of cultivation, the better the antibacterial film's activity. As shown in Table 1, the OD600 values for various antibacterial films were in the following order: control > CA > CA/2%TiO2/1.5%ZnO > CA/2%TiO2/2%ZnO > CA/2%TiO2/2.5%ZnO. It is important to note that the nanocomposite film made up of CA+2% TiO2+2.5% ZnO had the lowest OD600. In contrast to other antibacterial films, this film performed particularly well after 12 hours of cultivation. These results clearly showed that TiO2 and ZnO have a greater ability to resist bacteria growth [38-43].

Table 1. Effect of CA and CA/TiO2/ZnO in E. coli and S. aureus growth curve.

| Film Code | TiO2 wt% | ZnO wt% | Optical Density (OD) @600nm |
|-----------|----------|---------|-----------------------------|
| Control   | -        | -       | 0.62 ± 0.05                  |
| CA000     | 0.0      | 0.0     | 0.53 ± 0.03606              |
| CA215     | 2.0      | 1.5     | 0.16 ± 0.03055              |
| CA220     | 2.0      | 2.0     | 0.14 ± 0.02082              |
| CA225     | 2.0      | 2.5     | 0.13 ± 0.0202               |

The results are means of three replicates; ±, standard deviation (SD).

3. Conclusion
The present work employed the casting process to make ZnO-TiO2/CA composite films, which were then characterized. The tensile test demonstrated that the ultimate tensile strength and elongation were increased at a fixed loading level of 1.5 percent ZnO-2 percent TiO2/CA; however, once the loading level was exceeded, the values of tensile strength and elongation began to decline. The interaction between CA and ZnO-TiO2 was established by their FTIR spectra. The inclusion of ZnO resulted in an increase in hydrophobicity, which was also observed. When compared to pure CA, the combination of CA/TiO2/ZnO exhibits increased antibacterial activity.
against Escherichia coli and Staphylococcus aureus. Because of the wettability characteristics and antibacterial activity of the produced films, they have the potential to be employed in packaging applications.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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