Synthesis and characterization of zinc-titanium oxide nanoparticles blended methylcellulose derived from Albizia tree as an antibacterial natural polymer composite.

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Keywords: cellulose, polymeric, methylcellulose, nano titanium, nano zinc oxide, Albizia.

ABSTRACT

This paper seeks to synthesize antibacterial green polymeric composites utilizing extracted cellulose, a natural polymer sourced from biomass. The cellulose was obtained from local agricultural waste. Subsequently, the polymeric composites were synthesized, incorporating blends of nano zinc oxide and nano titanium dioxide in varying proportions. All synthesized samples undergo comprehensive characterization and examination through a range of tests encompassing structural and thermal tests. Furthermore, the antibacterial activity of the samples was assessed using the disc diffusion method, enabling the identification of the most effective formulations. The ultimate selection of the optimal composite was based on the collective outcomes of all tests, facilitating its potential utilization across diverse applications. In conclusion, the Albizia tree demonstrates a notable potential for efficient cellulose extraction, making it a viable candidate for such purposes. The cellulose extracted from Albizia tree holds the capability to yield methylcellulose. The bacterial activity assessment of Nano materials highlighted a distinct efficacy in the case of the 75% Nano TiO2 and 25% Nano ZnO mixture. However, it is worth noting that an excessive increment in Nano ZnO content exhibited adverse effects. Thorough thermal analyses verified the thermal stability of methylcellulose.
تحضير وتوصيف جزيئات أكسيد الزنك والتيتانيوم النانوية المخلوطة بمثيل السيلولوز المستخلص من شجرة البيزيا كمركب بوليمري طبيعي مضاد للجراثيم

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قسم علم الحياة، كلية العلوم، جامعة أورمية، إيران.

الملخص
تهدف هذه الورقة إلى توليف مركبات بوليمرية خضراء مضادة للبكتيريا باستخدام السيلولوز المستخرج، وهو بوليمر طبيعي مستخدم من الكتلة الحيوية. تم الحصول على السيلولوز من نفايات زراعية محلية. فيما بعد، تم توليف المركبات البوليمرية، بما في ذلك حلقات من أكسيد الزنك النانوي وثاني أكسيد التيتانيوم النانوي بلغت متوسطة مقاومة. خضعت جميع العينات المخلوطة وتوصيف وجود شامل من خلال مجموعة من الاختبارات تشتمل التحاليل البيئية والميكانيكية والموكلология. علاوة على ذلك، تم تقييم النشاط مضاد للتكتيريا للعوائق باستخدام طريقة اختبار الأصقال، مما سمح بتخفيض الكتربكات الأكثر فعالية. تم اختبار المركب البوليمرية استنادًا إلى التقييم الجماعي لجميع الاختبارات، مما يسهل استخدامه في مختبرات متنوعة. تم توجيه تسميته التربة بواخره ريشي ورشين، وهو بوليمر بيولوجي ملائم للمشروعات النباتية، مما يجعله مرشحًا قبلًا للتحقيق لحل هذه الأعراض. المستخرج من شجرة البيزيا يحمل القدرة على إنتاج الميثيل سيلولوز. أبرز تقييم النشاط البكتيري للمواد النانوية فعالية متميزة في حالة مزيج 75% من ثاني أكسيد التيتانيوم النانوي و 25% من أكسيد الزنك النانوي. ومع ذلك، يُذكر أن زيادة مطرقة في محتوى أكسيد الزنك النانوي أظهرت راحة سلبية. قامت التجارب الحرارية الشاملة بالتحقيق باستخدام الجلود بسياليوم، محصورة ضمن نطاق درجات حرارة أقل يتم درجة حرارة الخرافة. يُمكن أن تكون بعض الخصائص الميكانيكية، كالمادة المحمولة، بإضافة المواد النانومترية، فإن الخصائص الميكانيكية العامة لا تزال مرضية، خاصة في تطبيقات مثل العينة والتغليف.

الكلمات الدالة: سيلولوز، بوليمر، ميثيل سيلولوز، نانو تيتانيوم، نانو أكسيد الزنك، البيزيا.

1. INTRODUCTION
Over the course of numerous years, scientists have dedicated their endeavors towards discovering medications, vaccines, and alternative medical therapies to treat patients. However, the approach to curing illnesses has evolved over time, transitioning to novel concepts that revolve around eliminating disease by eradicating the microorganisms responsible for pandemics. This is achieved through the application of potent chemical disinfectants to sterilize various tools, surfaces, machines, and more. Although these efforts initially yielded promising outcomes, recent developments have unveiled concerns. These disinfectants, which were once regarded as highly effective, have now been identified as significant sources of pollution in water, alongside the discharge of drug residues. Natural cellulose is mainly contained material in plants as a result of photosynthesis process. This renewable material forms the base of many industrial products collected from tree, cotton, and other multicellular plants. Glucose as the monomer of this long chain linear polysaccharide binds each unit by β-1,4- glycoside linkage. In nature, bacteria, algae, and other soil creatures are capable to synthesis this fraction. As a cell part and carbon major source in structure and energy supplying for higher and micro-organisms is mostly in complex state with lignin, protein, pectin, and hemicellulose in varied ratio depending on the essential source

In general, nanocellulose in composites may be working as a dispersed filler in the target matrix with good chemical compatibility regardless to hydrophobicity of the other polymeric components. Coated layers of nanocellulose is well known technology in food packaging to increase / decrease resistance to related environmental determinations like moisture or water wetting angle, oxygen transmission, petroleum products, air constituents, …etc. [2; 3]. Recent works evaluated packing and coating fields with nanocellulose composites by basing on chemical and physical characterizations particularly water and oxygen enhancement [4-6]. Also, aerospace, energy, sensing and motion tools and equipments were another place of cellulose composite to propose a solution of future challenges [7; 8]. The qualifications of the formed layers especially chemical reactivity between coated layer – surface and surrounding atmosphere as well as hygroscopicity mainly depend upon substrate or coated surface, additives and applied method [9 - 11].
Suspensions of cellulose–titanium dioxide were prepared by various amount of titanium dioxide and characterized by FTIR, XRD, SEM, Atomic Force spectroscopies beside contact angle analyzer for water wettability. TiO$_2$ had a good influence on surface morphology where crystallinity raised as addition of this filler increased (more 84%) [12]. The research focused on investigating the thermal behavior of cellulose-metal oxide composites through depolymerization steps occurring below 375°C. Various metal oxides, namely ZnO, TiO$_2$, MgO, Cu/CuO, and magnetic fluid CoFe$_2$O$_4$, were utilized in the synthesis of these composites for different applications. Ex situ preparation route was chosen by several researchers to get ZnO nanoparticles then addition step to the biopolymer base. Here, homogenized hybrid features may be obtained with strong interaction between positively zinc ions in ZnO nanoparticles and negatively oxygen in hydroxyl linked to the polymeric cellulose chains. This strong interaction may enhance antibacterial activity against E. coli, P. aeruginosa, C. freundii, or S. aureus found in burn wounds [13-16].

2. Materials and Methods

2.1. Materials

Table 1 shows chemicals that used in this works with general information.

<table>
<thead>
<tr>
<th>Name</th>
<th>General specification</th>
</tr>
</thead>
</table>
| Albizia Lebbek (plant)   | Kingdom: Plantae- Clade: Mimosoid clade  
Genus: Albizia  
Species: A. lebbeck  
Family: Fabaceae |
| Sodium hydroxide         | NaOH, ≥98%, white, pellets, anhydrous, Sigma- Aldrich, USA.                             |
| Sulfuric acid            | H2SO4, 89%, Central Drug House (CDH®) Ltd., India.                                     |
| Titanium dioxide         | TiO2, white powder, nanoparticle, nanopowder, (10-30) nm, 99.5%, Skyspring Nanomaterials, Inc., USA. |
| Zinc oxide               | ZnO, white to light yellow, nanoparticle, nanopowder, (10-60) nm, 99.8%, Skyspring Nanomaterials, Inc., USA. |
| Sodium chlorite          | NaClO$_2$, white powder, 99%, Aldrich, Sigma- Aldrich, USA                             |
| dimethyl sulfate         | DMS, (CH$_3$O)$_2$SO$_2$, colorless oily liquid, CDH, India                           |
| Acetic acid              | CH$_3$COOH, colorless liquid, CDH, India                                              |

2.2. Characterization Instruments

Various instrumental techniques were used for sample characterization as below:

Fourier transform infrared (FTIR), 8400S, Shimadzu, Japan. Prepared methylcellulose and its composites were analyzed by Fourier transform infrared spectroscopy to define and compare their chemical structures where each spectrum was recorded in the range (4000 to 400) cm$^{-1}$ at transmittance mode as a wave number function.
Thermogravimetric Analysis (TGA), DTG-60, 100V, (50-60)Hz, 1000 VA, Shimadzu, Japan. TGA was used to evaluate thermal stability of the prepared materials where each sample was loaded in the platinum pan with heat range (30 to 600)°C / heating rate (10°C /min.) under the nitrogen atmosphere. Thermogram describes temperature and sample weight relationship due to thermal degradation and DTG graph as a derivative mode.

Differential Scanning Calorimetry Analysis was done to detect the thermal phase transition of methylcellulose nanocomposites. The tests were done using DSC analyzer (DSC-60/ Shimadzu/ Japan). The thermal range of test was (RT-300 °C)

2.3. Experimental Sets

The experiment work was designed into four stages as below:

2.3.1 Cellulose extraction:

Albizia Lebbek leaves and branches were collected then dried in air for several days to reach complete dryness. The dried leaves were grinded then sieved by using sieve with size 320 µ. Albezia powder (one gram) was mixed with 3% wt./v. alkaline solution (sodium hydroxide NaOH, 20 mL) at 60 °C and stirred mechanically for three hours to get rid of lignin and other water-soluble extractives. The resulted suspension was left standing for 24 hrs. Then, centrifugal washing and separation steps were done till natural pH was obtained. The obtained wetted substance was dried at 105 °C for 2 hrs. then the dried powder was bleached with aqueous sodium chlorite (3% wt/v) solution at 90 °C for 4 hrs to get rid of lignin and other phenolic residues. Finally, the bleached mixture washed with water then followed by centrifugal separation by centrifuge. The separated wet powder was dried at 106 °C for 2 hours. The extraction was fulfilled according to extraction method escribed in [17].

Fig. 1. Albizia tree and its collected leaves and branches

2.3.2 Methylation of cellulose

The methylation of extracted cellulose was done according the method described in [18]. Cellulose (1.0 g) was treated with aqueous caustic alkali (50% NaOH solution) for one hour at room temperature. The excess of this aqueous caustic alkali solution was taken out then dimethyl sulfate (3 mL) was added to produce sample. The mixture was occasionally stirred while it was in a water bath at 50 °C for three hours, being. In the final stages, the resulted material was neutralized by acetic acid solution (10%), filtered off on a sintered crucible, washed with acetone and dried at 50 °C for six hours.
I. Pure methylcellulose thin film

The composite samples was manufactured using film casting. Pure methylcellulose (without additives) was dissolved in distilled water (1 gram/100 mL) at room temperature. The final formula was poured into petri dish (8 cm diameter) plates, left to dry for 24 hours and kept in the desiccator till characterization tests [19].

II. Nanocomposite of Methylcellulose/nanometal oxides as thin films

This formula was obtained after repeating methylcellulose dissolving step in water as mentioned previously. Mixture nanoparticles were sonicated in distilled water by ultrasonic bath for 20 minutes in order to get homogenous dispersion solution then gradual addition of methylcellulose solution. The final mixture (1 gram of methyl cellulose and 1 gram of nano (TiO₂ and ZnO) mixture) was poured into petri dish (8 cm diameter) plates and left to dry to form thin film after twenty four hours[20].
2.3.3 Antibacterial Test:

Antibacterial activity was investigated as described in [20]. Resistance to *Escherichia coli* (*E. coli*) is a crucial aspect of effectively managing infections caused by this Gram-negative bacterium. The commonly employed method for evaluating this resistance is the disk diffusion technique, also known as the Kirby and Bauer method. In this standardized testing approach, an agar plate is inoculated with a suspension of *E. coli*. A disk containing the tested material is placed on the agar surface, allowing the bacteria to simultaneously grow and diffuse. The growth or inhibition of the bacteria occurs based on the effectiveness of the tested antimicrobial composite over a defined period (24 hours) and at a specific temperature.

In this context, the antimicrobial activity of nanocellulose and its composites was evaluated using Gram-negative bacteria, specifically the *Escherichia coli* ATCC 25922 strain. These bacterial strains were sourced from the Laboratory of the Directorate of Environment and Water Research and Technology, Ministry of Science and Technology in Baghdad, Iraq, through the agar disc method. Under sterile conditions, the nanocellulose and its composites were incubated at a temperature of 37 °C for 24 hours. The resulting inhibition zone, where bacterial growth was either present or suppressed, was then measured and documented. The size of this growth or inhibition zone was determined by the circular area of bacterial growth around the disk at the established inhibitory concentration.

3. Results and Discussion

Cellulose nano metal oxides composites were selected as the main targets in this work. Plant-based methylcellulose and its composites were obtained from natural source via traditional and methylation methods. The characterization tests results as shown in below:

3.1 Fourier Transform InfraRed (FTIR) Spectroscopy

FTIR spectra of the prepared cellulose and its nanocomposites are presented in Figures (3- 5) respectively. In these spectra, O-H and C-H stretching vibrations were (3600-2700) cm\(^{-1}\) while (1800-600) cm\(^{-1}\) stretching vibrations revealed fingerprint region. It can be noticed a broad peak of hydroxyl group (O-H) at 3341 cm\(^{-1}\), 2820 cm\(^{-1}\) for methyl and methylene stretching vibrations, C=O stretching vibration of carboxyl and acetyl at 1564 cm\(^{-1}\), while (1461 and 1485) cm\(^{-1}\) related to C-H, C-O band vibrations known as bending and stretching vibrations respectively.

Other distinguishing vibrations were deformation of C-H at 885 cm\(^{-1}\), stretching of C-O-C at 1153 cm\(^{-1}\), (663, 1622) cm\(^{-1}\) can be characterized as out-of-plane C-OH and OH in the bending respectively.

All FTIR spectra confirmed absence of lignin and hemicellulose in the samples related to 1730 cm\(^{-1}\) wavenumber. Intermolecular and intramolecular hydrogen bonding gave the prepared materials more selective crystallinity due to pendant hydroxyl groups that might be esterified by sulfuric acid hydrolysis and hydrogen bond network might be disordered.

Obtained composites revealed a weak vibration peak around 550 cm\(^{-1}\) related to the presence of ZnO nanoparticles [23], while a prominent vibrational peak related to Ti-O mode appeared around 1380 cm\(^{-1}\)[24].
Thermal analysis assessment of natural nanocomposites:

DSC and TDA/DTA tests were done to detect the thermal stability and phase transitions of nanocomposites. The results of tested are shown in below:

TGA/DTA thermal test:

These TFA/DTA thermograms Figures (6-9) showed several thermal stages with remarkable notes. Thermal decomposition of pure methylcellulose began degradation at 299 °C because of alkali –methylation influence on raw cellulose surface. These reactants affected quantity of methyl group attachment beside residual ionic hydroxyl on the surface. This ionic hydroxyl formed by NaOH may cause a deformed distribution. Also, high temperature range indicated an extraordinary stabilized nano-shaped structure of methylcellulose. It is significant note that endothermic peak at 383.71 caused high changing in DTA behaviour as a transformation between decomposition - recrystallization cellulose phases[25, 26, 27].Table 2 summarized the results of this test.
Table 2. TG-DTA analysis of the prepared materials.

<table>
<thead>
<tr>
<th>Material identity</th>
<th>Thermal stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure methylcellulose</td>
<td>T, °C</td>
<td>46.64</td>
<td>299</td>
<td>350.51</td>
<td>383.71</td>
<td>501.07</td>
</tr>
<tr>
<td></td>
<td>Yield, %</td>
<td>114.009</td>
<td>108.131</td>
<td>576.936</td>
<td>-629.644</td>
<td>-626.668</td>
</tr>
<tr>
<td>Cellulose with (25% TiO2 + 75% ZnO)</td>
<td>T, °C</td>
<td>41.82</td>
<td>270.16</td>
<td>369.26</td>
<td>501.19</td>
<td>-</td>
</tr>
<tr>
<td>nanocomposite</td>
<td>Yield, %</td>
<td>100.241</td>
<td>96.131</td>
<td>35.415</td>
<td>26.168</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose with (50% TiO2 + 50% ZnO)</td>
<td>T, °C</td>
<td>37.13</td>
<td>159.58</td>
<td>281.33</td>
<td>382.54</td>
<td>499.29</td>
</tr>
<tr>
<td>nanocomposite</td>
<td>Yield, %</td>
<td>101.525</td>
<td>99.234</td>
<td>97.574</td>
<td>49.572</td>
<td>45.46</td>
</tr>
<tr>
<td>Cellulose with (75% TiO2 + 25% ZnO)</td>
<td>T, °C</td>
<td>35.74</td>
<td>172.08</td>
<td>285.40</td>
<td>378.67</td>
<td>502.46</td>
</tr>
<tr>
<td>nanocomposite</td>
<td>Yield, %</td>
<td>100.21</td>
<td>98.718</td>
<td>97.427</td>
<td>49.989</td>
<td>45.322</td>
</tr>
</tbody>
</table>

Differential Scanning Calorimetry (DSC) Test:

DSC experiments showed varied exothermic and endothermic thermal transformation[28, 29].

- Pure methyl cellulose showed an endothermic event at 260.43°C after heat intake (114.75 J/g) while exothermic point was with (-168.45 J/gm).
In pure methylcellulose, enthalpy change (ΔH) between exothermic and endothermic points clarified a heat gain with 53.69 J/g.

Nano-metal oxide mixture showed a semi constant exothermic range in peak with increasing of TiO₂ (or decreasing of ZnO). ZnO that had a remarkable rising in temperature then decreased with to (75% TiO₂ + 25% ZnO).

More heat release in exothermic transformation phase after addition of nano metal oxides mixture to.

Formed nanocomposites showed less number of endothermic modes of heat (or enthalpy) compared to exothermic mode.

Required heat for endothermic mode after nano metal oxide was less compared to free sample (114.76 J/g) except (25%TiO₂ & 75ZnO) composite (189.47 J/g) where enthalpy difference (ΔH) = 74.71 J/g.

This significant note revealed to the effect of composite formula and effect of mixing performance leading to the formation pores inside composite structure.

Addition of 75% TiO₂ & 25% ZnO gave one thermal transition pointed at less peak temperature compared to methylcellulose with less ΔH (-111.06 J/g).

This important note was repeated with 25%TiO₂ and 75% ZnO with ΔH varying: (168.45 – 77.32) = 91.13 J/g meaning more heat gain with less TiO₂ and higher ZnO addition.

Equal quantity of both nano metal oxides presented a little distinguishable change in heat or temperature related to exothermic phase behaviour.

In endothermic behaviour of these tested sample, addition of 25% TiO₂ & 75% ZnO caused a rise in heat compared to zero addition of nano metal oxide (189.47 – 168.45 = 21.02 J/g) while equal quantity of both nano additives gave (168.47 - 62.49 = 105.98 J/gm).

It is a noticeable note that temperatures of all endothermic points after nano metal oxide addition were close to pure methyl cellulose temperature (260.43 °C).

Addition of nano metal oxide mixture to nano-methylcellulose presented thermal stable materials.

The results of this test are listed in Table 3 and figures 10 and 11. The results shows that

Table 3. DSC results of the prepared materials.

<table>
<thead>
<tr>
<th>Sample identity</th>
<th>Peak, °C</th>
<th>Onset, °C</th>
<th>Endset, °C</th>
<th>Heat, J/gm</th>
<th>Peak Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure methylcellulose</td>
<td>92.95</td>
<td>58.62</td>
<td>120.28</td>
<td>-168.45</td>
<td>Exothermic</td>
</tr>
<tr>
<td></td>
<td>260.43</td>
<td>231.63</td>
<td>299.24</td>
<td>114.76</td>
<td>Endothermic</td>
</tr>
<tr>
<td>Nanocellulose + (25% TiO₂ + 75% ZnO)</td>
<td>86.26</td>
<td>76.30</td>
<td>112.92</td>
<td>-77.32</td>
<td>Exothermic</td>
</tr>
<tr>
<td></td>
<td>248.56</td>
<td>222.91</td>
<td>297.44</td>
<td>189.47</td>
<td>Endothermic</td>
</tr>
<tr>
<td>Nanocellulose &amp; (50% TiO₂ + 50% ZnO)</td>
<td>96.78</td>
<td>63.27</td>
<td>123.12</td>
<td>-169.29</td>
<td>Exothermic</td>
</tr>
<tr>
<td></td>
<td>253.13</td>
<td>229.90</td>
<td>213.65</td>
<td>62.49</td>
<td>Endothermic</td>
</tr>
<tr>
<td>Nanocellulose + (75% TiO₂ + 25% ZnO)</td>
<td>89.60</td>
<td>51.34</td>
<td>115.90</td>
<td>-111.06</td>
<td>Exothermic</td>
</tr>
</tbody>
</table>
Biological Activity:

The assessment of bacterial activity holds significant importance within current research endeavors. As depicted in Figure 14, it is evident that the sample comprising a blend of 75% TiO$_2$ and 25% Nano ZnO achieved the most substantial inhibition zone. Specifically, Figure 14 highlights that the maximum inhibition zone measures approximately 14mm. This outcome serves as compelling evidence, signifying the success of this particular mixture within the scope of this study. Conversely, alternative concentrations of the same mixture, as illustrated in Figures 11 -12, resulted in more moderate or weak responses...

The most significant component in increasing the antibacterial activity of TiO$_2$ NPs is to manage their shape and crystal structure. Besides, the antibacterial mechanism of TiO$_2$ is different from ZnO. The first one depends on photocatalytic method to kill or inhibit bacterial that the germicidal mechanisms of TiO$_2$ nanoparticles involve the discharge of positively charged ions into the reaction media, which is linked to the (negatively charged) thiol group (-SH) of proteins on the cytoplasmic membrane. This interaction causes cell wall capture and increased permeability, as well as deformation of the structure of cellular components such as DNA, ribosomes, and cellular enzymes, and eventually death of the microbial cell [30].
As a result of membrane lipid peroxidation, ZnO NP raises the levels of reactive oxygen species (ROS) and malondialdehyde in bacterial cells. NPs degrade the permeable membrane, denature intracellular proteins, damage DNA, and cause membrane leakage. Based on these findings, the action of ZnONPs has been attributed to the fact that broad-spectrum antibacterial action against -lactam-resistant Gram-negative food pathogens is mediated by Zn\textsuperscript{2+} ion-induced oxidative stress, which results in depletion, -lactamase enzyme inhibition, intracellular protein inactivation, DNA damage, and eventually cell death\cite{31}.

As it is noticed from the bacterial inhibition yest, the activity of ZnO is weak compared with aTiO2 activity as explained before. The main reason of this difference can be attributed to differences in particle size of two materials which is played crucial role of specifying antibacterial activity because the surface area is increased when the particle diameter is small leading to large exposed area against bacteria. According to manufacturer, the TiO2 particle diameter is between (10-30)nm while ZnO particle diameter (10-60)nm. The activity changes because of this variance in particle sizes of both and that is consistent with results of \cite{32,33}.

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Table 4.6 Escherichia coli inhibition zone of cellulose composites.

<table>
<thead>
<tr>
<th>TiO₂, %</th>
<th>ZnO, %</th>
<th>Inhibition zone, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>14</td>
</tr>
</tbody>
</table>

5. CONCLUSIONS

The Albizia tree demonstrates a notable potential for efficient cellulose extraction, making it a viable candidate for such purposes. The cellulose extracted from Albizia tree holds the capability to yield methyl cellulose. The bacterial activity assessment of nano materials highlighted a distinct efficacy in the case of the 75% Nano TiO₂ and 25% Nano ZnO mixture. However, it is worth noting that an excessive increment in Nano ZnO content exhibited adverse effects. Thorough thermal analyses verified the thermal stability of methylcellulose, particularly within a lower temperature spectrum encompassing room temperature.

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