Disinfectant natural rubber films filled with modified zinc oxide nanoparticles: Synergetic effect of mechanical and antibacterial properties

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Abstract. Disinfectant natural rubber (NR) film filled with nano-zinc oxide (ZnO_{np}) was prepared *via* latex processes. In addition, modification of ZnO_{np} was done by coating calcium carbonate (CaCO_3) at the ratios of 90:10 and 60:40 ($\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$). Mechanical and thermo-mechanical properties, together with the unique anti-microbial activity of the resulting NR film products, were studied in detail. It was found that the nature of ZnO dispersion plays an important role in the improvement of the properties of NR films. Enhancement in the properties of NR was noticed for the films by the addition of $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and a reduction in properties was observed in the case of unmodified ZnO_{np} . Van-der Waals force of attraction among the ZnO_{np} particles and the improved degree of crosslinking of NR molecules are the reasons for the property enhancement. The results are well correlated with qualitative and quantitative determinations against gram-negative *E. coli* anti-microbial studies. Usage of modified- ZnO_{np} effectively kills bacteria through the formation of ROS and Zn^{++} , which transfer across the NR molecules *via* electrostatic forces. Hence, it can be effectively used in the dipping process to develop gloves, condoms, clothes etc.

Keywords: nanocomposites, rubber, material testing, mechanical properties, anti-microbial properties

1. Introduction

Natural rubber (NR) is a widely used material in various industrial applications. Due to its excellent elasticity and mechanical properties, NR has been used in the manufacturing of tires, sports articles, sealing materials, gloves, rubber boots, and dairy rubber items [1]. In order to reach the specific properties of products, the addition of several fillers such as carbon black, silica and clay were incorporated. Fillers affect the intrinsic properties of NR significantly;

- Changes the insulating NR to a conducting elastomer for electronic devices [2–5],
- Increases abrasion resistance and wet traction of NR used in car tires [6–9],
- Improves the hydrophilic nature of NR used as organic substrates [10, 11].

NR has been used in various fields, but it is not suitable for medical applications due to its allergenic nature and inability to disinfect the microorganism. Hence, the anti-microbial property of NR has to be

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improved by incorporating inorganic particles with high surface area in contact with NR matrix. This can be achieved by the incorporation of several nano-particles such as silver (AgNP) [12–14], titanium dioxide (TiO₂) [15, 16], and zinc oxide (ZnO) [17–22] to disinfect microbes such as *E. coli*, *Staphylococcus aureus*, *Salmonella typhus*, *Pseudomonas sp.*, *Salmonella sp.*, *Shigella sp.*, *K. pneumonia* etc. The investigation was initiated by loading AgNP in thermoplastic low-density polyethylene (LDPE) and polypropylene (PP) as anti-microbial composite films against *E. coli* and *S. aureus*, which showed a 99.9% decrease in the number of viable bacteria [23]. Similarly, polysulfone membranes (PSf) containing AgNP exhibited a decrease of 90% *Escherichia coli* adhered cells compared to the pure PSf [24]. However, it was found that the disinfecting efficiency of the composites has strongly related to the nature of dispersion of AgNP, which cannot be easily controlled due to its high surface area and strong Van der Waals force of attraction among AgNP, resulting in agglomeration during film preparation [25]. Also, AgNP is toxic and harmful to humans on a short-/long-term exposure owing to oxidative and inflammatory infections [26]. Therefore, the usage of AgNP should be minimized.

ZnO nanoparticle was found to be an inorganic metal oxide to replace the toxic AgNP. It is a nontoxic metal oxide and also a safe anti-microbial agent generally used in medical devices and preservative packaging applications [27]. In addition, ZnO is recognized as a multi-functional material to fabricate polymer nanocomposite with exceptional electrical, optical, thermal, mechanical, catalytic, and biomedical properties [28]. ZnO can distort and damage the surface of bacterial cells, bringing about spillage of intracellular substances and finally removes the bacterial cells [28]. It was found that ZnO effectively reacts with light and moisture and consequently produces hydrogen peroxide (H₂O₂) to kill bacteria [17]. ZnO dispersed in poly(ether ether ketone) (PEEK) showed superior antibacterial activity against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacteria [29]. Incorporating ZnO into natural polymers such as ZnO-based natural rubber (NR) foam was assessed by *S. aureus* and *E. coli* antibacterial vulnerability tests. The study on the antibacterial activity of the foam composites revealed that the activity of nano-sized ZnO has been

extended to the NR foam [30]. This causes several advantages to the NR foam products. This technology can also be applied for the preparation of NR film by the addition of nano- and micro-ZnO in pre-vulcanized NR latex. The result clearly showed the effectiveness in antifungal activity of the film with nano-ZnO compared to the one with micro-ZnO at the same concentration [31]. However, the use of ZnO in NR for performing disinfection is still challenging since it is difficult to disperse ZnO in the hydrophobic NR matrix. Therefore, study in this area is limited and most of the NR film products exhibit poor ability to resist bacterial and micro-organism. When considering ZnO reinforced into NR, it can be used as an activator in NR products by reacting with stearic acid to form zinc stearate, which effectively propagates crosslinking among the NR molecules [32]. This means that the use of ZnO in NR film acts as both an activator and anti-microbial agent. However, the formation of bonds of Zn–O–Zn among particles results in strong agglomeration of ZnO [33], which reduces the properties of ZnO-filled NR, and the modification of ZnO might be needed to avoid agglomeration.

Therefore, in the present work, ZnO incorporated NR composites were prepared by varying the loading levels of 0–7 phr. In addition, the variation of properties of NR on the particle size, surface area, and doses of ZnO was investigated for different types of ZnO. White seal ZnO (ZnO_{ws}), ZnO nanoparticles (ZnO_{np}) and ZnO_{np}-coated with CaCO₃ (ZnO_{np}-CaCO₃) at the %weight ratios of 90:10 and 60:40 (ZnO: CaCO₃) were incorporated into NR. The modification of ZnO_{np} with CaCO₃ aims to improve the dispersion of ZnO_{np} inside the NR matrix in order to enhance crosslinking propagation of NR molecules and also the disinfection degree of the film. Mechanical and dynamical properties, thermo-mechanical properties, thermal stability, and biological testing of the resulting composites were investigated. The main aim of this work is to develop new medical disinfection goods to be used as rubber-glove, shoes, masks, and personal protective equipment (PPE) with NR as the main component. Also, the disinfection nature of these ZnO particles was elucidated at different degrees of loading throughout the NR matrix. Modification of ZnO_{np} with CaCO₃ was minimized the cost of additives effectively since it reduces the purity of ZnO_{np} and significantly improves the properties.

2. Experimental

2.1. Materials

Natural rubber (NR) latex with high ammonia concentration was manufactured by Chalong Latex Industry Co., Ltd. (Songkhla, Thailand). Various types of ZnO particles used in this work are as shown in Table 1, including ZnO_{ws} (white seal grade) together with different types of ZnO nanoparticles (*i.e.*, ZnO_{np} and ZnO-adsorbed CaCO₃ at the ratios 90:10 (ZnO_{np}-Ca₁₀) and 60:40 (ZnO_{np}-Ca₄₀)). All ZnO_{np} were produced through wet preparation processes [34] and supplied by Global Chemical Co., Ltd. (Samut Prakan, Thailand). In addition, the potassium hydroxide was manufactured by Qingdao Hisea Chem Co. Ltd., (Qingdao, China). Potassium Laurate, 50% zinc diethyldithiocarbamate (ZDEC), 50% poly(dicyclopentadiene-*co-p*-cresol), and 50% sulfur were supplied by Thanodom Trading Co. Ltd. (Bangkok, Thailand).

2.2. Preparation of NR/ZnO composite films

NR latex was compounded according to the formulation shown in Table 2. The compounding process was initiated by the addition of specific chemicals (Table 1) into NR latex using a mechanical stirrer at

Table 1. Specification of each ZnO used in the present study provided directly by Global Chemical Co., Ltd. using BET analysis.

Ingredients	ZnO content [%]	Specific surface area [m ² /g]
ZnO _{ws}	99.50	5
ZnO _{np}	97.83	40
ZnO _{np} -Ca ₁₀	91.39	40
ZnO _{np} -Ca ₄₀	60.92	40

Table 2. Formulation for NR latex compound with different types of ZnO.

Ingredients	Contents [phr]
60% NR latex	100
20% Potassium Laurate	1.00
10% Potassium hydroxide	0.25
50% Zinc diethyldithiocarbamate (ZDEC)	1.25
50% Poly(dicyclopentadiene- <i>co-p</i> -cresol)	1.00
50% Sulfur	2.00
25% ZnO _{ws}	0, 3, 5, 7
25% ZnO _{np}	
25% ZnO _{np} -Ca ₁₀	
25% ZnO _{np} -Ca ₄₀	

150 rpm. The mixing was continued at room temperature for 24 h the so-called maturation of NR latex compound. Thereafter, the NR/ZnO composite films were prepared by using the latex mixing method, and the ingredients are as shown in Table 2. The mixing process was started by stirring the latex compound for 3 min before the addition of ZnO. Mixing was continued for another 10 min and dipped a glass plate inside the latex compound for 10 s to get the films with a thickness of approximately 1.0–1.5 mm. Samples were eventually dried at 70 °C for 24 h to form the NR/ZnO composite films. It is noted that, during the drying propagation for 25% of cure time, the sample was again dipped into a 5 wt% ZnO dispersion with controlled dipping times periodically up to 24 h. Finally, the films were removed from the mold, washed with distilled water, and kept in the desiccator. The total ZnO concentrations were controlled at 3, 5, and 7 phr after the cleaning process of the samples through %weight evaluation. Thus, only 90% of ZnO was added in each individual formulation during compounding. Another 10% was incorporated eventually before performing the test. It is noted that all the composite films were labeled as NR/normal ZnO, NR/ZnO_{np}, NR/ZnO_{np}-Ca₁₀, and NR/ZnO_{np}-Ca₄₀ for the NR composites filled with normal ZnO, ZnO nanoparticle, and ZnO_{np}-coated CaCO₃ at ZnO:CaCO₃ ratios of 90:10 and 60:40, respectively.

3. Characterizations

3.1. Particle size analyzer

ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀, and ZnO_{np}-Ca₄₀ were initially dispersed in deionized water at 0.5 wt%. The suspension of each ZnO was evaluated by using the dynamic light scattering (DLS) technique with the help of a nanoparticle analyzer, SZ-100 series (HORIBA Instruments (Singapore) Pvt Ltd, Changi Business Park Vista, Singapore).

3.2. Mechanical properties

Mechanical properties of pure NR and NR composites filled with different types of ZnO were investigated using a universal tensile testing machine (Tinius Olsen model 1 ST, Tinius Olsen, Co., Ltd., Salfords, UK). The tests were carried out with a cross-head speed of 500 mm/min at room temperature, according to ISO 37.

3.3. Crosslink density

Crosslink densities of samples were determined from swelling experiments in toluene. The samples with dimension $(10 \times 10 \times 2)$ mm³ were prepared and weighed before immersing into toluene under ambient conditions for seven days following the ASTM D471-79. The swollen samples were then removed, and the excess liquid on the surface was removed by blotting with filter paper. These specimens were dried in a hot air oven at 60 °C until attaining a constant weight. The final weight was then compared with the original weight, and the apparent crosslink density was eventually calculated using the Flory-Rehner equation (Equation (1)) [35]:

$$\nu = \frac{-(\ln(1 - \phi_p) + \phi_p + \chi \phi_p^2)}{V_1(\phi_p^{1/3} - \phi_{p/2})} \quad (1)$$

where ν is the crosslink density [mol/m³], ϕ_p is the volume fraction of rubber in a swollen network, V_1 refers to the molar volume of toluene, and χ is the Flory-Huggins interaction parameter (0.4) between toluene and NR [36].

3.4. Temperature scanning stress relaxation (TSSR)

Stress relaxation of pure NR, NR/ZnO_{ws}, NR/ZnO_{np}, NR/ZnO_{np}-Ca₁₀, and ZnO_{np}-Ca₄₀ was investigated with a TSSR meter (Brabender GmbH & Co. KG, Duisburg, Germany). The dumbbell-shaped specimens were first prepared according to ISO 527, type 5A. The specimens were annealed at 100 °C for 30 min, cooled down to room temperature, and conditioned for about 30 min before testing. This was to avoid interference from the storage hardening of NR due to non-rubber constituents [37]. The test was performed by extending the sample at a constant strain of 50% and conditioning at 23 °C for 2 h. Then, the non-isothermal test was performed by raising the temperature from 23 to 220 °C at a constant heating rate of 2 °C/min. The observed results were used to explain the relationship between stress relaxation and relaxation spectrum as a function of temperature.

3.5. Thermogravimetric analysis (TGA)

Thermal analysis of pure NR and its composites was performed by using thermogravimetric analyzer TGA STA8000 (Perkin Elmer, Ohio, USA). The measurement was carried out under nitrogen atmosphere in the temperature range of 30 to 600 °C before switching to

oxygen atmosphere with a holding time of 5 min and raised the temperature to 800 °C at a heating rate of 10 °C/min.

3.6. Morphologies

The morphologies of unmodified and modified ZnO particles with CaCO₃ were characterized with transmission electron microscope (TEM) model JEOL JEM-1400 (JEOL, Tokyo, Japan) using 80 kV accelerating voltage. In addition, morphological properties of the composites were studied by using optical microscopy (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Also, the high-resolution morphologies and the energy dispersive X-ray analysis (EDX) patterns of the composites were obtained from a scanning electron microscope (SEM) (Apreo, Thermo Fisher Scientific, Brno, Czech Republic). The samples were first cryogenically fractured in liquid nitrogen to create a new cross-sectional surface. Then, the surface was sputter-coated with a thin layer of gold under vacuum before scanning by SEM.

3.7. Anti-microbial properties

Anti-microbial properties of gum NR, NR/ZnO_{ws}, NR/ZnO_{np}, NR/ZnO_{np}-Ca₁₀, and ZnO_{np}-Ca₄₀ composites were characterized by means of qualitative and quantitative determinations. The qualitative determination was performed to observe the clear zone near the sample. First, Luria Broth medium was prepared, and a single colony of the Gram-negative *E. coli* bacteria was inoculated under aseptic conditions. Then, it was incubated at 37 °C for 12 h in a mechanical shaker. Subsequently, 100 µl of a 10⁸ cfu/ml bacteria suspension was spread onto agar plates. Then, gum NR and its composites were cut in circular shape with a diameter of 6 mm and sterilized by 70% ethanol. The samples were eventually placed onto agar plates and aerobically incubated at 37 °C for 24 h in an incubator. The diameter of the zone of inhibition can be measured and reported on a centimeter scale [30].

The quantitative determination was carried out by optical density with a spectrophotometer (Thermo Fisher Scientific Genesys 20 (4001/4) Spectrophotometer, Apeldoorn, Netherlands) at 600 nanometer wavelength. In addition, the initial bacteria suspension of *E. coli* was prepared at an optical density of 0.2 ($OD_{600} = 0.2$). The samples of gum NR, NR/ZnO_{ws}, NR/ZnO_{np}, NR/ZnO_{np}-Ca₁₀, and NR/ZnO_{np}-Ca₄₀ composites were prepared with a dimension of

$10 \times 10 \text{ mm}^2$. Consequently, the samples were placed in a 50 ml tube of bacteria suspension and then incubated at 37°C in a mechanical shaker for 1, 2, and 4 h for optical density testing by spectrophotometer.

4. Result and discussion

4.1. Effect of modification of ZnO on the observed particle size

Figure 1 shows the particle size distribution of ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ obtained from the particle analyzer. It is observed that the polydispersity index (PDI) of ZnO_{ws} is 0.336, while the

Figure 1. Particle size distribution of ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$.

values of $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$, and ZnO_{np} are found to be 0.355, 0.354, and 0.335, respectively. This reveals that all samples provided a narrow range of particle size distribution, indicated better dispersion of particles. In addition, the histogram shows the particle size ranges from 2 to $20 \mu\text{m}$ for ZnO_{ws} . It is also found that the ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ showed particle size in the range of 100 to 1000 nm. This confirms the smaller particle sizes of ZnO_{np} than the normal one. It is also seen in Figure 1 that the largest particle is observed in ZnO_{ws} with an average particle size of $7.697 \mu\text{m}$. However, $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$, and ZnO_{np} exhibited smaller average particle size than that of ZnO_{ws} at 193.48, 171.25, and 218.6 nm, respectively. Additionally, $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ exhibited the smallest average particle size when compared to $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and ZnO_{np} . It is clearly seen that the modification of ZnO by absorbing CaCO_3 reduces the average particle sizes of ZnO_{np} , and this can be the reason for better dispersion of ZnO_{np} throughout the NR matrix during preparation. These results are comparable with the TEM images of ZnO_{ws} (Figure 2a) and ZnO_{np} particles, which show ZnO aggregates as represented in Figure 2. It is clearly seen that the size of ZnO_{np} (Figure 2b) reduces upon its modification with CaCO_3 as well as on increasing the concentration of CaCO_3 (Figures 2c, 2d).

Figure 2. TEM images of ZnO_{ws} (a), ZnO_{np} (b), $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ (c) and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ (d).

4.2. Relation between mechanical properties and apparent crosslink density

Figure 3 shows stress-strain curves of NR films filled with ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$. Also, the details of 100%moduli, tensile strength, and elongation at break are summarized in Figure 3. A considerable change in the stress-strain curves is observed after the addition of ZnO, as shown in Figure 3a. This relates to the strain-induced crystallization (SIC) behavior of the vulcanizates by increasing the crosslink density of the NR matrix. Thus, the slope (m) of the curve is significantly increased due to the presence of ZnO. On comparing the types of ZnO, ZnO_{np} showed the lowest SIC of 0.03364, which might be owing to the degree of ZnO_{np} agglomeration and related to its high surface area, increasing the opportunity of ZnO to contact together rather than react with the NR compounds. However, the agglomeration of ZnO_{np} is reduced by the coating of CaCO_3 on ZnO_{np} , and it leads to an increase in the slope to 0.09226 and 0.08446 for $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$, respectively. Considering Figure 3b, it is obviously seen that the tensile properties of the gum NR are significantly increased upon the addition of all types of ZnO enhances the crosslinking ability by the activation of ZnO inside the NR matrix.

As seen in Figure 3a, the vulcanizate with ZnO_{np} exhibited a strong reduction of tensile strength compared to other types of ZnO. However, concerning the addition of ZnO, $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$, a slight difference is observed in the tensile properties. Here, it is indicated that the highest tensile strength is observed after the modification of ZnO_{np} with CaCO_3 due to the improved ZnO dispersion inside the NR matrix, particularly the one filled with $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$. States of dispersion and distribution of each ZnO inside the NR vulcanizate based on the indication of tensile properties as displayed in the proposed model of Figure 4. Here, the large particle of ZnO_{ws} has a low specific surface area leading to lower interaction between its particles which reduces particle agglomeration and also activates the vulcanization of the sulfur curing system (Figure 4a). However, the lowest tensile strength observed in NR/ ZnO_{np} vulcanizate is due to the small particle size in the order of nanometer, which generated high specific surface area and supported high Van der Waals force between particles, leading to agglomeration of ZnO_{np} as observed in Figure 4b. However, for the one filled with modified ZnO_{np} , i.e., $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$, improved dispersion is observed due to the obstruction of Van der Waals force of attraction among

Figure 3. Stress-strain curves of NR films filled with 5 phr of ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ (a), and the observed tensile strength, 100%modulus and elongation at break of NR films with 0, 3, 5 and 7 phr of ZnO (b).

Figure 4. Proposed dispersion model of NR films filled with ZnO_{ws} (a), ZnO_{np} (b), $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ (c) and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ (d).

ZnO_{np} particles by the existence of CaCO_3 [38]. It is also clearly observed in Figure 3 and as shown in the proposed model (Figures 4c and 4d). The excess CaCO_3 of 40 wt% lowers the ability of crosslink formation in NR molecules and reduces the tensile strength of NR films filled with $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ compared to $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$. Conversely, the values of moduli and elongation in the case of the films filled with different types of ZnO, there is no considerable effect on the addition of 5 phr ZnO since the concentration is not high enough for providing reinforcement. Thus, the dispersion of ZnO and ZnO_{np} is the key factor to exhibit different film properties according to the different free volumes inside the matrix. This can be explained by the tensile properties of vulcanizates, as shown in Figure 3b. It is noted that the increase of each ZnO up to 7 phr does not change much in the tensile strength. However, a strong decrease in tensile performance is clearly observed in the case of ZnO_{np} due to the strong ZnO agglomeration within the NR matrix.

It indicates that the tensile properties of NR vulcanizates are effectively related to the nature of dispersion of ZnO throughout the matrix since it controls the origination of crosslinking among NR molecules. In order to clarify the occurrence of crosslinking propagation in the vulcanizates, the reduced stress as a function of extension ratio is taken into account by using the Mooney–Rivlin equation (Equation (2)) [39], and the relation is plotted as shown in Figure 5.

Figure 5. Mooney–Rivlin plot of NR films filled with ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$.

$$\sigma^* = \frac{\sigma}{\lambda - \frac{1}{\lambda^2}} = 2C_1 + \frac{2C_2}{\lambda} \quad (2)$$

where σ and λ are the stress and the extension ratio according to the tensile testing, respectively, whereas C_1 and C_2 are the parameters related to the crosslink density and entanglement of NR molecular chains, respectively. According to James and Guth approach, the reduced stress (σ^*) refers to the elastic modulus, which is proportional to the crosslink density of the vulcanizates [39]. Thus, the crosslink density of each vulcanizate was fitted from Equation (2) corresponds to the observed $2C_1$ in connection with the reduced stress as a function of the reciprocal of λ in

Table 3. Comparison of crosslink density determined from Mooney–Rivlin and Flory–Rehner equation.

Samples	Mooney–Rivlin equation	Flory–Rehner equation [mol/m ³]
Gum NR	0.0050	24.51
NR/ZnO _{ws}	0.0094	58.58
NR/ZnO _{np}	0.0089	50.62
NR/ZnO _{np} -Ca ₁₀	0.0011	88.44
NR/ZnO _{np} -Ca ₄₀	0.0096	65.14

Figure 5. The observed crosslink density is in good agreement with the tensile properties, particularly the tensile strength of the vulcanizates. Here, the addition of all types of ZnO increases significantly the crosslinking of the NR matrix, while the highest value is noticed for the NR film filled with ZnO_{np}-Ca₁₀. However, according to the agglomeration of ZnO_{np}, poor crosslinking propagation is found and leads to poor tensile properties, as shown in Figure 4 and Table 3.

Chemical crosslinks present in the NR vulcanizates filled with ZnO can also be evaluated by using the swelling method according to the Flory–Rehner equation. Figure 6 shows the crosslinking density of NR films, and a similar trend is observed as seen from the Mooney–Rivlin equation, and the crosslink density is found to be increased with the addition of ZnO. Here, the gum NR exhibited the lowest crosslink density at 24.51 mol/m³, whereas the addition of unmodified and modified ZnO increases crosslinking of the gum NR at approximately 100%. As expected, this

Figure 6. Crosslink density of NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

relates to the activation of ZnO to the vulcanization propagation of the NR molecules. Although, ZnO_{np} shows poor crosslinking capability due to the strong agglomeration of ZnO. Its modification with CaCO₃ provided significant improvement in the molecular chain crosslinks, as seen in NR vulcanizates filled with ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀. The occurrence of chemical crosslinking of NR molecular chains through sulfur vulcanization system can be explained from the proposed model as shown in Figure 7. During the processes of curing at 70 °C, ZnO particles generate vulcanization [40]. Here, the Zn⁺⁺ forms a complex molecular structure with the ZDEC molecules in order to activate sulfur for beginning crosslinking propagation from the S_x and S_y radicals [40], as shown in Reaction (A) of Figure 7. Then, the breakages of sulfur radicals react individually with the NR molecular chains, performing chemical crosslinking of mono-, di- and polysulphidic linkages, as shown in the proposed Reaction (B) of Figure 7. This means that the degree of crosslinking of NR molecular chain depends on the amount of Zn⁺⁺ release from ZnO particles. For different types of ZnO

Figure 7. Proposed vulcanization model of NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

in the present work, the formation of chemical crosslinking is mainly due to two reasons: (i) ZnO dispersion and (ii) purity of ZnO (Table 1). Considering Figure 7 to explain the effect of ZnO dispersion, it is found that the highest amount of Zn^{++} releases in the case of the NR films filled with ZnO_{np} -Ca₁₀ and ZnO_{np} -Ca₄₀. The lowest amount of such ions is formed in the composite with ZnO and ZnO_{np} . It can be related to their degree of dispersion, which causes different specific surface areas [31, 40]. Therefore, poor crosslinking density is observed in the composite of uncoated ZnO particles. However, due to the less purity of ZnO_{np} in the film with ZnO_{np} -Ca₄₀, the highest crosslink density is observed in NR/ ZnO_{np} -Ca₁₀ film. In addition, the dispersion can be clearly confirmed from the morphologies of vulcanizates using SEM-EDX analyses, as shown in Figure 8. It is observed that the high level of agglomeration of ZnO occurs in the vulcanizate with ZnO_{np} (Figures 8d–8f), whereas the one with both modified

ZnO_{np} showed homogenous dispersion of the ZnO particles and Zn^{++} . It is noted that the NR films with ZnO_{np} -Ca₁₀ indicated better dispersion of ZnO throughout the NR matrix (Figures 8g–8i)) and it shows the highest crosslink density of the vulcanizate. Strong agglomeration of ZnO_{np} is also observed in the OM images, as shown in Figure 9. A smooth surface on the NR film is observed after the addition of ZnO, ZnO_{np} -Ca₁₀, and ZnO_{np} -Ca₄₀, while a roughened surface is observed in the film with ZnO_{np} . Also, the agglomeration of ZnO_{np} makes the composites stronger when the ZnO loading is increased from 3 to 7 phr. This is the result of decreased tensile strength of the NR film on increasing the amount of ZnO in the NR matrix. Concerning the purity of ZnO present in each composite as displayed in Table 1, ZnO_{ws} and ZnO_{np} are highly purified, and the lowest one is found to be ZnO_{np} -Ca₄₀. Theoretically, ZnO_{ws} and ZnO_{np} are expected to generate the highest Zn^{++} and lead to exhibit the highest crosslink

Figure 8. SEM micrographs and its EDX mapping of NR films filled with ZnO_{ws} (a–c), ZnO_{np} (d–f), ZnO_{np} -Ca₁₀ (g–i) and ZnO_{np} -Ca₄₀ (j–m).

Figure 9. Light microscope images of NR films filled with ZnO_{ws} (a–c), ZnO_{np} (d–f), $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ (g–i) and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ (j–l) with concentrations 3, 5 and 7 phr, respectively.

density than the other vulcanizates. However, higher level of agglomeration and larger particle sizes of the ZnO_{ws} , the vulcanizates filled with $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ have reached the optimum cross-linking ability of NR matrix and provided the highest tensile properties with homogenous dispersion of ZnO_{np} throughout the NR matrix. This is a useful finding beneficial to NR industries to minimize the amount of ZnO in the NR matrix with better mechanical properties and crosslink density.

4.3. Relation between thermal stability and thermo-mechanical relaxation

In order to measure the thermal stability of the vulcanizates filled with different types of ZnO and ZnO_{np} , TGA thermograms and their specific details by means of the decomposition temperature (T_d) and %residual are presented in Figure 10 and Table 4. TGA thermograms reveal that the thermal decomposition under nitrogen (N_2) atmosphere of NR vulcanizates with and without ZnO_{ws} and ZnO_{np} is found at approximately 370 °C, corresponding to the

decomposition of the NR matrix. However, considering the decomposition temperature range of 350–400 °C in the inserted image (i) of Figure 10, a slight difference of 1.0–1.5 °C in the T_d value is observed upon using ZnO_{ws} and unmodified ZnO_{np} although they have prepared by different procedures. Here, the ZnO_{ws} is produced by French process based on combustion of zinc at 1230–1270 °C [41], while ZnO_{np} and its modification have been done based on a wet chemical process with spontaneous reaction at 154 °C [34]. This ZnO_{np} normally has poor thermal resistance than the one with the French process [41]. However, upon modification with CaCO_3 , the T_d of the film has no significant differences from the one with ZnO_{ws} . This might be due to the improved dispersion of the ZnO_{np} particles throughout the rubber matrix. Negligible thermal stability of the NR film with ZnO_{ws} is due to the benefit of combustion process can also be elucidated through the %residual composition as shown in the inserted image (ii) of Figure 10. Here, the white residue of the film with ZnO_{ws} is the remaining ZnO_{ws} powder. A lower

Figure 10. TGA (a) and DTG (b) thermograms of NR films filled with ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$.

Table 4. Decomposition temperatures (T_d) and % residue of NR films filled with ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$.

Samples	Decomposition temperature, T_d [°C]	%Residue
Gum NR	377.50	6.09
NR/ ZnO_{ws}	378.85	6.89
NR/ ZnO_{np}	377.50	6.39
NR/ $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$	378.50	5.53
NR/ $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$	378.33	4.87

%residual is observed after using ZnOnp and its modified forms due to those nano-particles have lower purity. It is noted that the different amount of residue in each film can be considered related to (i) the remaining ZnO or ZnOnp and (ii) the carbon black based on decomposition of hydrocarbon NR molecules. Thus, due to the lower NR content and purity of ZnOnp in cases of $\text{ZnOnp}\text{-Ca}_{10}$ and $\text{ZnOnp}\text{-Ca}_{40}$, the residues of pure NR are higher than the composites films. This means that the NR films exhibited significant improvement in thermal stability together with lowering the non-environmental friendly components.

However, the thermal stability of the vulcanizates has been completely changed after measuring the thermo-mechanical stability using the recently developed technique TSSR. Here, the vulcanizates are stretched and recorded the stress relaxation on raising

the temperature until it reaches the decomposition state. For the determination of stability, the temperatures at which the stress relaxation is reduced to 50 and 90% (T_{50} and T_{90}) are explored [42]. Figure 11a shows the stress relaxation as a function of temperature for the NR vulcanizates filled with different types of ZnO_{ws} and ZnOnp . Also, Table 5 summarizes the observed T_{50} , T_{90} , initial stress relaxation (σ), and crosslink density of the samples. It is interestingly noted that the highest thermo-mechanical stability of the vulcanizates is found in the one filled with $\text{ZnOnp}\text{-Ca}_{10}$, which replaces the ZnO_{ws} as displayed in the TGA. This means that the degradability of the vulcanizates has been changed after the extension of 50% strain. This is attributed to the SIC of the NR molecular chain which has initially formed as the crystallinity during extension of the samples based on the rearrangement of NR chains. Thus, NR/ $\text{ZnOnp}\text{-Ca}_{10}$ film, which contains homogenous dispersion of ZnOnp exhibits higher stability, showing T_{50} and T_{90} 1.36 and 4.08% higher than the other vulcanizates. On the other hand, as expected, the NR film filled with ZnOnp showed the lowest thermo-mechanical stability compared to the other ZnO -based vulcanizates due to the agglomeration of particles. Therefore, it summarizes that the NR vulcanizate filled with $\text{ZnOnp}\text{-Ca}_{10}$ exhibited the highest capability to retard thermal degradation under an applied force.

Figure 11. Stress relaxation (a) and relaxation spectra (b) of NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

TSSR not only indicates the thermal stability of the vulcanizates but also provides σ , which generally relates to the 50% modulus of the samples. The tensile strength values of the NR vulcanizates, as seen in Figure 3, are in good agreement with the σ values. Vulcanizates filled with ZnO_{np}-Ca₁₀ provided the highest σ value, followed by the one with ZnO_{np}-Ca₄₀, ZnO_{ws}, and ZnO_{np}. This is due to the homogeneous dispersion of modified ZnO_{np} throughout the NR matrix, which effectively increases crosslinking propagation among the NR molecular chains and finally retards the breakages of the samples during extension. Figure 11a shows the stress relaxation is increased with increasing temperature approximately up to 50–60 °C related to the entropy effect of the NR molecular chain. This behavior can be used to estimate the crosslink density (ν) of the vulcanized samples through TSSR by Neo–Hookean equation (Equation (3)) [43]:

$$\sigma = \nu \cdot R \cdot T \left(\frac{\lambda}{1 + \alpha(T - T_0)} - \left(\frac{\lambda}{1 + \alpha(T - T_0)} \right)^2 \right) \quad (3)$$

where λ is the constant extension ratio, α is the thermal expansion coefficient ($2.43 \cdot 10^{-4} \text{ K}^{-1}$), R is the universal gas constant, and σ is the stress at a reference temperature. Also, T and T_0 are the reference-, and the initial temperatures of the measurements. Table 5 depicts the crosslink density ν of the vulcanizates, and it shows the lowest value, approximately 62.06 mol/m^3 , for the gum NR. An effective increase

Table 5. TSSR results in terms of σ_0 , T_x and ν of NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

Samples	σ_0^* [MPa]	T_{50}^{**} [°C]	T_{90}^{**} [°C]	ν^* [mol/m ³]
Gum NR	0.20	123.50	150.20	62.06
NR/ZnO _{ws}	0.42	145.70	163.20	147.37
NR/ZnO _{np}	0.39	141.00	159.60	107.80
NR/ZnO _{np} -Ca ₁₀	0.45	147.60	165.30	212.17
NR/ZnO _{np} -Ca ₄₀	0.43	141.20	160.50	110.74

* σ_0 and ν are the initial stress at starting temperature and crosslink density.

** T_{50} and T_{90} are the temperature at which the forces were decreased to 50 and 90% from σ_0 .

in ν of NR can be seen by the addition of ZnO_{ws}. However, this value is found to be lowered upon the incorporation of ZnO_{np} due to its poor dispersion. By reducing the interaction among ZnO_{np} by absorbing CaCO₃, the crosslink density is significantly increased in the case of NR/ZnO_{np}-Ca₄₀ and NR/ZnO_{np}-Ca₁₀ vulcanizates. A considerable increment in the ν value is achieved for the NR/ZnO_{np}-Ca₁₀ and correlated the results very well with the values indicated in Mooney–Rivlin and Flory–Rehner evaluations (Table 3). It clearly clarifies that the crosslink density of the vulcanizates has strongly related to ZnO_{ws} and ZnO_{np} dispersion, and therefore the one with ZnO_{np}-Ca₁₀ showed the highest value even it consists of ZnO, approximately 10% lower than the ZnO_{ws}.

In addition, the thermo-mechanical degradation of stress relaxation temperature curves in Figure 11a shows a strong decomposition beyond 120 °C. This can be explained by the decomposition of polysulfidic bonding among NR molecular chain which is evaluated by differentiating the non-isothermal relaxation modulus ($E(T)$) at a constant heating rate ($\nu = 2^\circ\text{C}/\text{min}$) using the Equation (4). This calculation provides the equation for relaxation spectrum ($H(T)$) as a function of temperature:

$$H(T) = -T \left[\frac{dE(T)}{dT} \right]_{\nu = \text{const}} \quad (4)$$

Figure 11b shows relaxation spectra of gum NR, ZnO_{ws} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$, and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ -filled NR films. Relevant peaks are indicated in the temperature range of 100–180 °C, which is directly assigned to the decomposition of $\text{-C-S}_x\text{-C-}$ chemical crosslinks in the NR matrix [44] designated as peak β . Therefore, the height of the peak can be interpreted as the existing conventional crosslinking density of the samples. In Figure 11b, it is seen that gum NR showed the lowest intensity of peak β , which means the lowest $\text{-C-S}_x\text{-C-}$ bonding, resulting in low σ and mechanical properties together with poor T_{50} and T_{90} . For the same reason, the incorporation of ZnO_{np} resulted in the low peak height compared to the other NR vulcanizates filled with different ZnO_{ws} and ZnO_{np} due to the poor dispersion of ZnO_{np} throughout the NR matrix. On the other hand, the addition of $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ effectively increases the peak intensity. This means that the vulcanizate has optimal crosslink degrees due to the generation of $\text{-C-S}_x\text{-C-}$ chemical bonds. As expected, this correlates to the state of ZnO_{np} dispersion which can act as the activator for NR molecules developed bonding among allylic carbon atoms within the NR molecular chains. It is also seen that the

incorporation of $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ has also exhibited higher peak intensity than the one with ZnO_{np} even it consists of approximately 60% ZnO_{np} . Therefore, all the results are well correlated with the properties observed for the NR films related to mechanical properties (Figure 3), initial stress relaxation (Figure 11) and the crosslink density via Mooney–Rivlin plot (Figure 5), swellability (Figure 6) and TSSR (Figure 11).

4.4. Antibacterial properties

The incorporation of modified- ZnO_{np} has been strongly affected the properties of NR films in terms of their mechanical, dynamical, and thermal stability based on the nature of dispersion of ZnO particles. However, ZnO has also exhibited good performance due to its excellent anti-microbial properties. To study the benefit of using NR films, NR vulcanizates filled with ZnO_{ws} , ZnO_{np} , and modified ZnO_{np} are introduced to evaluate their disinfection ability by means of qualitative and quantitative determination against Gram-negative *E. coli* which has been reported as the most common type of bacteria causing infection [45], including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), traveler's diarrhea and also other clinical infections such as neonatal meningitis and pneumonia [46, 47]. As a result, the qualitative clearing zone or inhibition zone after the bacterial infection is examined, as seen in Figure 12. It is observed that the controlled NR film, which is an NR vulcanizate without ZnO showed no clear zone. It clearly indicates that the gum NR has no ability to suppress the *E. coli* bacteria. In contrast, the clearing zone can be clearly observed after the addition of ZnO into the NR matrix. This indicates the antibacterial performance of the films by creating the reactive oxygen species (ROS) and solubilized zinc (II) ion (Zn^{++}) to destroy the bacteria's cell wall [48], as

Figure 12. The qualitative anti-microbial activities against Gram-negative *E. coli* of NR films filled with ZnO_{ws} , ZnO_{np} , $\text{ZnO}_{\text{np}}\text{-Ca}_{10}$ and $\text{ZnO}_{\text{np}}\text{-Ca}_{40}$ at the loading of 3 (a), 5 (b) and 7 phr (c).

Figure 13. Proposed model of anti-microbial mechanism of NR films filled with ZnO. a) Photocatalytic process of ZnO with light and moisture and b) attacking of ROS and Zn ions to the bacteria.

shown in the proposed model (Figure 13). Although the disinfection mechanism of pure ZnO_{np} has not been proven yet and several proposed models have been provided by researchers from various fields. However, based on the interpretation mechanism of ZnO_{np}-filled NR vulcanizates, there is no prior clarity in the explanation. Figure 13a shows the first proposed mechanism originated from the physical and chemical interactions of the ZnO or ZnO_{np} surfaces with light and moisture. This causes ROS absorption on ZnO_{np} surfaces by means of superoxide anion ($\text{O}_2^{\bullet-}$) (Figure 13a_i) and hydroxyl radicals (•OH) + proton (H^+) (Figure 13a_{ii}). These are originated from the reaction of positive holes (h^+) in the valence band (VB) and free electrons (e^-) in the conduction band (CV) of the ZnO_{np} to the moisture molecules (H_2O) and oxygen gas (O_2), respectively [49–51]. It is noted that the •O_2^- is further reacted with H^+ and forms •HO_2 . Later on, this product is found to be interacted with H^+ and e^- before generating the hydrogen peroxide (H_2O_2), as shown in (Figure 13a_{iii}). This means that the ZnO_{np} surface consists of •O_2^- , •OH and H_2O_2 . However, the negative charges of •O_2^- and •OH cannot penetrate into the negative charges of bacteria membrane, and the H_2O_2 molecules have the access and make injuries. Finally, it destroys the cells and triggers cell death due to the destruction of cellular components such as lipid, protein, and DNA (Figure 13b_i). This phenomenon leads to the dysfunctionality of membrane and cell wall of bacteria, resulting in oxidative stress towards the bacteria relating internalization of the ZnO_{np} particles into the cell and further the microbe cannot be survived. However, during internalization into the cytoplasmic area of bacteria, it has not only from the ZnO_{np}, but

also from the solubilized Zn^{++} present in the rubber matrix as well as the dissolution of ZnO_{np} with the medium including bacteria (particularly the cytoplasm which consists of 80% water) [52–55], as shown in Figure 13b_{ii}. With the electrostatic interaction of the Zn^{++} and ZnO_{np}, disruption of DNA replication on the cellular of bacteria occurs due to the effect of active transport inhibition along with the amino acid metabolism and the disturbance of the enzyme system [56, 57], as observed in Figure 13b_{iii}. According to the mechanism summarized in the proposed model (Figure 13), attacking of ROS and Zn^{++} to the bacteria is strongly dependent on the active surface area of ZnO in order to increase the ability to generate active species on ZnO surfaces and also to enhance the penetration ability of ZnO particles and Zn^{++} into the cell. Therefore, the purity and particle size of ZnO during dispersion in the NR matrix need to be clarified. Comparing two different methods of adding ZnO into the NR matrix, as shown in Figure 14, indicated significant differences in the disinfection behavior. Figure 14a does not show the clear zone in NR films prepared directly by adding 5 phr of ZnO into NR without dipping processes. On the other hand, Figure 14b shows clear zones in the case of the samples prepared using the dipping procedure due to the killing of bacteria. This can be explained from the NR layers with different thickness covering on the ZnO particles (Figure 14c and 14d), which relates directly to the electrostatic transfer of ZnO particles and their active ion species. In addition, it has been reported that the photocatalytic activity of ZnO was reduced by incorporating into NR [58]. However, it has to be noted that the exact evaluation of the NR layer absorbed on the ZnO surfaces

Figure 14. The clear zone of NR films prepared by directly adding 5 phr of ZnO in the cases of without (a) and with (b) dipping processes together with their proposed models (c) and (d).

cannot be clearly detected through bound rubber layer determination and morphologies since the ZnO concentration is too less relative to the NR matrix. Thus, the model is interpreted based on the different results of anti-microbe degrees. From Figures 14c and 14d, the generation of e^- and h^+ has been occurred directly on the ZnO surfaces together with the solubilized Zn^{++} as a result of the interaction with the accelerator component in the NR film during the curing process. Here, such active ions can move across the NR layer by the electrostatic force with a negative charge of bacteria surfaces when the layer has a thickness lower than 4 nm [4, 59]. Therefore, in the case of using the dipping process, the thickness of the NR layer has lowered when compared to the one with a direct mixing process. Here, the interaction leads to the generation of ROS components and the movement of Zn^{++} into bacteria cellular have been well originated. This is the reason for providing a clear zone, as seen in Figures 12 and 14. However, this phenomenon has a strong relation with the degree of

dispersion of ZnO_{ws} and ZnO_{np} with and without modification. Agglomeration of ZnO might be the reason for lowering antibacterial activity of the films. Table 6 shows the observed diameters of the clear zone in case of each ZnO types at various concentrations. No significant differences are observed in the values except the films with ZnO_{ws} which has strong agglomeration of large ZnO particles. Interestingly, the indistinguishable qualitative results of the NR films filled with unmodified and modified ZnO_{np} by varying their contents are represented. This relates to the high surface areas of the fine dispersion of ZnO_{np} which is well active to generate ROS and Zn^{++} for penetrating the bacterial cells.

Compared to the qualitative analysis, only 5 phr of each ZnO type are selected for investigating the quantitative determination of bacterial activity. Characterization is performed by using the observed optical intensity of bacteria suspension based on *E. coli* (Gram-negative) received from the spectrophotometer at a wavelength of 600 nm (OD_{600}), and the results are as shown in Figure 15. Here, the OD_{600} is reported for the Luria Broth medium of *E. coli* that incubated at temperature 37 °C with each ZnO-filled NR film of 1, 2, 4, and 6 h using the initial bacteria suspension of 0.2 ($OD_{600} = 0.2$). It is revealed that the gum NR exhibited a considerable increase in OD_{600} as a function of time, indicating the growth of *E. coli* under controlled conditions. This confirms that there is no antibacterial activity against *E. coli*, and it is well related to the none qualitative clear zone (Figure 12). It

Table 6. Diameters of the clear zone for NR films filled with different types of ZnO at various concentrations.

Samples	Diameter [cm]		
	3 phr	5 phr	7 phr
NR/ ZnO_{ws}	1.00±0.10	1.10±0.90	1.00±0.85
NR/ ZnO_{np}	0.90±0.09	1.00±0.70	1.15±0.10
NR/ ZnO_{np} -Ca ₁₀	0.80±0.07	1.00±0.80	1.20±0.99
NR/ ZnO_{np} -Ca ₄₀	1.20±0.08	1.10±0.78	1.10±0.80

Figure 15. Optical density of bacteria suspension *E. coli* (Gram negative) by spectrophotometry at a wavelength of 600 nm for NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

is seen in Figure 14 that OD_{600} decreases upon the addition of 60 % ZnO_{np} to the NR film filled with ZnO_{np}-Ca₄₀. Liberation of Zn⁺⁺ and H₂O₂ occurs and reacts effectively with the microbe cell at the membrane and cytoplasm regions. This is also the result of non-increasing of OD_{600} on the incorporation of ZnO_{ws}, ZnO_{np} and ZnO_{np}-Ca₁₀. The results show no significant differences at 6 h of test; even they have different ZnO purity. This means that the addition of modified ZnO_{np}, especially ZnO_{np}-Ca₁₀, exhibited better dispersion which causes a different degree of liberation of Zn⁺⁺ and H₂O₂ to attack the microorganism. It has the benefit of lowering the usage of ZnO in the NR film, which minimizes the non-environmental friendly material while the properties are significantly improved along with antibacterial performance.

The activity of bacteria can also be elucidated through the degree of inhibition by Equation (5):

$$\% \text{ inhibition } (\omega) = \frac{N}{N_0} \cdot 100 \quad (5)$$

where N_0 and N are the mean values of bacteria concentrations from OD_{600} on the gum NR and its films with different ZnO types, respectively.

Figure 16 shows the %inhibition of bacteria activity in NR film with and without ZnO for 6 h. It is seen that the values of ω show as almost constant after incubating for 1 h. However, the values are found to be drastically increased beyond the incubating period of 1 up to 2 h, and slightly increases during 4–6 h for all the films using ZnO_{ws} and ZnO_{np} with and without modification. Here, the different types of

Figure 16. % Inhibition bacteria activity of NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀ and ZnO_{np}-Ca₄₀.

ZnO provided distinct values of ω . The NR film filled with ZnO_{np}-Ca₄₀ showed a lower value than the films filled with ZnO_{ws}, ZnO_{np}, and ZnO_{np}-Ca₁₀. This is mainly due to the different percentages of ZnO used in each film. As seen in Table 1, ZnO_{np}-Ca₄₀ consists of the lowest ZnO purity of 60%, whereas the ZnO_{ws}, ZnO_{np}, and ZnO_{np}-Ca₁₀ composed the ZnO of approximately 99, 97, and 90%, respectively. With a 10% difference in the ZnO content, the NR film filled with ZnO_{np}-Ca₁₀ showed comparatively better results. This means that the particle size and the dispersion of ZnO are played the most important role in the anti-microbial activity of NR films. Modification of ZnO_{np} with CaCO₃ clearly exhibited an effective improvement in the properties of NR films compared to the ZnO_{np}, which forms strong agglomeration during mixing with NR in latex states. Although there are no significant differences in the anti-microbial ability of the resulting composite, mechanical (Figures 3 and 5) and thermo-mechanical properties (Figure 11 and Table 5) are clearly improved. It proves well the effect of film processing and modification of ZnO_{np} for enhancing the properties effectively and upheaving the applications of NR films in the near future products.

5. Conclusions

NR films filled with ZnO_{ws}, ZnO_{np}, ZnO_{np}-Ca₁₀, and ZnO_{np}-Ca₄₀ were prepared via latex mixing technique. To determine the effect of surface area and degree of dispersion of ZnO, the mechanical properties, cross-link density, thermal stability, morphology, thermo-mechanical properties, and anti-microbial activity of films were studied. It was found that the nature of

dispersion of ZnO plays an important role in the improvement of crosslink density and mechanical properties of the films. Both the properties were improved considerably after the modification of ZnO_{np} surface with CaCO₃. This lead to hinder Van-der Waals force of attraction among ZnO_{np} particles, and the ZnO_{np} effectively activated the crosslinking propagation. The presented results were in good agreement with the observed relevant properties based on Mooney–Rivlin plotting, film swelling, and TSSR measurements. On the other hand, significantly poor properties were observed for the unmodified-ZnO_{np} reinforced NR due to the strong ZnO_{np} agglomeration. The anti-microbial activities in terms of qualitative and quantitative determination against Gram-negative *E. coli*, it was found that the incorporation of modified-ZnO_{np}, particularly ZnO_{np}-Ca₁₀, provided the effective killing of bacteria through the formation of ROS and Zn⁺⁺. The results concluded that the potentially key factor of the anti-microbial activity in the NR films was the surface area and degree of dispersion of ZnO_{ws} and ZnO_{np} with and without modification. It was observed that the optimal degree of anti-microbial activity could be achieved by using ZnO of less purity, while the other properties were found to be maximized. The current study based on the film processing and ZnO_{np} modification was highly beneficial to the NR film industries by improving the anti-microbial activity of the material together with better mechanical properties and cost-effectiveness, particularly in the case of NR gloves and medical NR-coated textiles. Upon the modification of ZnO with CaCO₃, dispersion of ZnO_{np} was effectively improved even though it has less purity. This is due to the high specific surface area of ZnO_{np}, which stimulates crosslinking of NR molecular chains and improves the anti-microbial properties. Less usage of ZnO in NR film is also important for minimizing the environmental pollution based on the decomposition of NR products since ZnO is considered a toxic constituent, particularly to aquatic organisms.

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