

<http://journal.rmutp.ac.th/>

Effects of Chitosan Contents on Latex Properties and Physical Properties of Natural Rubber Latex/Chitosan Composites

Siwarote Boonrasri^{1*} Pongdhorn Sae–Oui² Mathurot Chaiharn³
and Chananpat Rardniyom⁴

^{1,4} Faculty of Engineering and Agro-industry, Maejo University

63 Sansai-Phrao Road, Nongharn, Sansai, Chiang Mai, 50290

² MTEC, National Science and Technology Development Agency (NSTDA)

114 Phahonyothin Road, Klong Nueng, Klong Luang, Pathumthani, 12120

³ Faculty of Science, Maejo University

63 Sansai-Phrao Road, Nongharn, Sansai, Chiang Mai, 50290

Received 4 October 2017; Accepted 5 March 2018

Abstract

In this study, natural rubber latex/chitosan (NRL/CT) composites were prepared by latex mixing. The effects of chitosan on latex properties and physical properties of NRL/CT composite films were focused. It was found that there was no significant change in the latex properties with increasing CT content. Increasing CT contents of 0.5 to 8.0 phr caused the rising of 300 % modulus of the NRL/CT composite whereas the opposite trend was found for tensile strength and elongation at break. The antimicrobial efficacy of the chitosan-incorporated rubber was slightly enhanced from that of the non-chitosan rubber. By comprehensive consideration, CT content suitable for antibacterial gloves according to Thai industrial standards (TIS 1056-1: 2013) was 1 phr. For other latex products that require high modulus and antibacterial activity such as tubing, pillow foam, rubber nipple and catheter, CT could be used at higher contents from 2 to 4 phr.

Keywords: Natural Rubber Latex; Chitosan; Composite; Antibacterial Activity

1. Introduction

Natural rubber latex (NRL) is a colloid system having cis-polyisoprene particles dispersed in aqueous medium derived from the cytoplasm of laticiferous cells of *Hevea brasiliensis* plants [1]. Average sizes of the rubber particles in NRL are around 0.02 to 3.00 μm . The particles are covered by a layer of negatively charged proteins and lipids, which provides colloidal stability to the NRL [2]. NRL is white milky liquid that undergoes coagulation by acid (such as formic acid or other acids) or salt (calcium chloride, calcium nitrate, etc.) at room temperature [3]. The products from NRL such as tubing, pillow foam, rubber nipple, surgical glove and catheter find extensive applications in the biomedical field [4, 5]. The advantages of these products are their elasticity and flexibility [6]. However, the products from NRL show some weaknesses such as low modulus and poor tear resistance that affect the quality of the NRL products. Consequently, many attempts have been made to use reinforcing fillers such as modified montmorillonite [7], ultra-fine calcium carbonate [8], multi-walled carbon nanotube [9], graphene oxide [10], nano-silica [11], and starch [12] to improve NRL properties. The incorporation of reinforcing fillers from renewable resources into rubber can result in new composites with enhanced properties and biodegradability, so it is of interest to study the possibility of using chitosan

as a reinforcing filler.

Chitin is a structural polysaccharide and is the second abundant natural polymer after cellulose. Chitin is found in shells of arthropods (crabs, lobsters, shrimps, crayfish, king crabs, and insects) as well as mollusks (e.g., squid pens). Chitosan (CT) is a linear copolymer composed of β -(1-4)-linked N-acetyl-d-glucosamine obtained by partial chitin deacetylation normally found in the form of granules, sheets, or powders [13]. There are currently a variety of applications for chitosan in the biomedical, food, and chemical industries owing to its interesting properties, such as biodegradability, biocompatibility, low toxicity, and biological activity [14, 15]. CT has demonstrated good potential as an antimicrobial agent [16].

Several approaches have been reported for the preparation of composites comprising CT reinforced NR or epoxidized natural rubber (ENR), including CT acid solution mixed with NRL [17-21], and CT dry mixed by a 2-roll milling process [22]. For CT acid solution mixed with NRL, CT is dissolved completely to form an acidified aqueous media prepared by dissolving CT in distilled water containing 2% (v/v) acetic acid prior to mixing with NR latex containing curing agents. This method may cause poor dispersion of chitosan because latex particle surfaces are negative charges and, when the positive charges of the acid are

added, the rubber particles are quickly coagulated. In this work, chitosan was dispersed in water without acid. CT particles were prepared to have a negative charge that could be dispersed in water by using a ball mill. In view of this, we have focused on incorporating CT as a new type (dispersion form) of CT in NRL compounds. One of the most significant requirements for chitosan to be successfully employed as a filler is the good interaction with the rubber. Therefore, the effect of CT content on latex properties, tensile properties, morphology and antimicrobial efficacy of natural rubber latex/chitosan (NRL/CT) composites are investigated.

2. Research Methodology

2.1 Materials

High-ammonia (HA type) concentrated NRL with 60 % of dry rubber content (DRC) was manufactured by Thai Rubber Latex (Thailand). The concentrated NRL was stabilized by adding 0.7 % ammonium hydroxide solution. Chitosan oligomer from crab shells in a powder form having particle size of 100 mesh (149 μm), weight-average molecular weight of 2.7×10^4 g/mol, and deacetylation degree of about 85 % was obtained from Taming-enterprises (Thailand). The other compounding ingredients used were 10% potassium hydroxide (KOH) solution, 10% potassium oleate solution, 50% sulphur (S) dispersion, 50%

zinc diethyl dithiocarbamate (ZDEC) dispersion, 50 % zinc oxide (ZnO) dispersion, 50 % phenolic antioxidant (PA) dispersion, which were all purchased from Lucky Four Co., Ltd. (Thailand).

2.2 Preparation and characterization of 10 % Chitosan (CT) dispersion

The mixing of CT with other ingredients, as shown in **Table 1**, was performed by crushing and grinding CT using a ball mill for 4 days and then testing of 10 % CT dispersion was carried out. The pH and viscosity of the 10% CT dispersion were measured. The particle size of the CT dispersion was determined using a microscope.

Table 1 Ingredients for the preparation of 10% CT dispersion

Ingredients	% (w/w)
Chitosan	10.0
Bentonite clay	1.0
Vultamol	1.0
Water	78.0
Alkaline substance	10.0
Total	100.0

2.3 Preparation of NRL/CT compounds

The mixing of CT (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 phr) with NRL and other ingredients, as shown in **Table 2**, was performed using a latex mixing technique. Fine ball-milled dispersions with particle size less than 5 mm of the vulcanizing agents and the others were

added to the NRL. The mixture was then heated at 45°C in a mixing vessel for 24 h with an intermittent stirring and cooled down at room temperature for 24 h prior to testing.

Total solid content (TSC, %) of the latex compound was determined according to ISO 124. pH of the latex compound was measured by using a pH meter, conforming to ISO 976. The chloroform number (CN) test was performed through the coagulation of a sample via mixing with an equal volume of chloroform. After 2-3 min, the coagulum was examined and graded according to the texture of the coagulum. The chloroform number (CN) was expressed as follows: (1) unvulcanized, (2) lightly vulcanized, (3) moderately vulcanized, and (4) fully vulcanized.

2.4 Preparation of NRL/CT composite films

After maturation of latex compounds at 45 °C for 24 h, the latex compounds were cooled down at room temperature for 24 h. NRL/CT composite films were prepared by the dry coagulant dipping process. Cleaned glass tubes were dipped into a 20 % calcium chloride solution for 10 s and dried in an oven at 100 °C for 5 min. Then, the glass tubes were dipped into the NRL/CT latex compound for 1 min followed by drying in the oven at 110 °C for 40 min. The dried NRL/CT composite films were conditioned at room temperature for 24

h prior to being tested for their physical properties.

Table 2 Latex compound formula of NRL/CT composites

Materials	phr
60 % NRL (HA type)	100
10 % KOH	0.2
10 % Potassium oleate	0.5
50 % S	1.0
50 % ZDEC	0.75
50 % PA	1
50 % ZnO	2
10% Chitosan	Varied*

* 0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 phr

2.5 Determination of physical properties of NRL/CT composite films

Dumbbell shaped test pieces were cut from rubber film of NRL/CT composites. Tensile test was done in accordance with ISO 37 using a computerized tensile tester (Instron 5565) with load cell of 10 kN. The crosshead speed of the Instron machine was set at 500 mm/min. The modulus at 300 % elongation (300% Modulus), tensile strength and elongation at break (EB) were analyzed from the tensile data. The values of tensile properties were the average of 5 measurements.

Morphology of the NRL/CT composites was studied by using a field emission scanning electron microscope (FE-SEM, model S-4700, Hitachi, Japan) at 5 kV electron energy. The newly

cryogenic fractured surfaces, coated with Pt-Pd, of the NRL/CT composites were examined.

2.6 Antimicrobial activity of NRL/CT composite films

Antimicrobial studies were done by the agar diffusion method [23]. The NRL/CT composite films were cut with the dimensions of 10 mm × 10 mm and placed on Mueller Hinton agar medium (Merck, Germany) which had been previously seeded with 10⁵ cfu/ml (Colony Forming Units/mL) of inoculums containing the tested bacterium using *S.aureus*. The plates were then incubated at 37 °C for 24 h. The antimicrobial properties of the NRL/CT composite films were expressed by the inhibitory effect on microbial growth based on the growth inhibition zone diameter (in cm) or clear zone diameter around the composite films.

3. Results and Discussion

3.1 Properties of the 10% CT dispersion and morphology of the NRL/CT composites

The CT used in this experiment was in a form of small flake and soluble in acetic acid. However, we cannot dissolve CT with acid because it will cause coagulation when the CT solution is added into NRL. Therefore, CT must be transformed into a dispersion form before being added into latex. It was

found that after the addition of 10 % CT dispersion, the rubber particles in the latex did not precipitate.

The pH of the 10% CT dispersion was 10.26 which was close to that of the latex (**Table 3**), therefore 10% CT dispersion could be incorporated into NR latex without coagulation. Based on the theory that CT normally has a positive charge, but it may be changed into a negative charge at high pH value due to the presence of the alkaline substance in the formula (**Table 1**). Hence, it was successful for preparing 10% CT dispersion.

Fig. 1 depicts microscopic images of CT in different forms. It was found that the particle size of commercial chitosan was very large with the sizes between 50.0 and 100.0 µm (**Fig. 1A**). However, the particle size of the chitosan in the CT dispersion prepared by ball mill was significantly lower with the particle sizes between 1.0 and 5.0 µm (**Fig. 1B**). This indicates that CT particles could be broken down during the ball mill mixing.

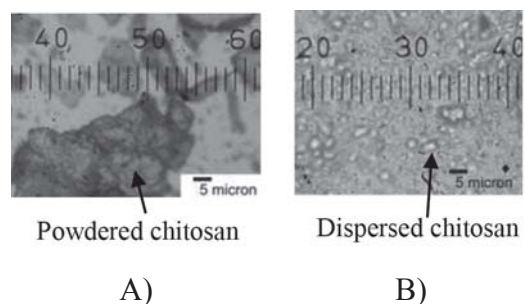


Fig. 1 Images from the microscope of
A) commercial chitosan,
B) chitosan in the CT dispersion

Fig. 2A-B show the SEM micrographs of the NRL/CT composites containing 4.0 phr of CT taken at magnifications of 20k and 40k, respectively, and **Fig. 2C-D** depict the SEM micrographs of the NRL/CT composites containing 8.0 phr of CT taken at magnifications of 20k and 40k, respectively. The CT particles could be seen more clearly at higher magnifications. The group of white spots and high ridges represented the CT particles (indicated by the arrows) and the grey areas were the NR matrix. In addition, it could be clearly seen in **Fig. 2D** that the size of CT particles (shown in **Fig. 1A**) was reduced to approximately 1.0-2.0 μm after ball mill mixing.

It was clear from the micrographs that a fine and uniform phase distribution was exhibited in the NRL/CT composites containing 4.0 phr of CT. With increasing CT content to 8.0 phr, the fractured surfaces showed poorer dispersion and, thus, a larger size of CT particle (see **Fig. 2D**). From the SEM results, there was no formation of holes or voids at the interface between rubber and CT indicating the good wetting between rubber and CT.

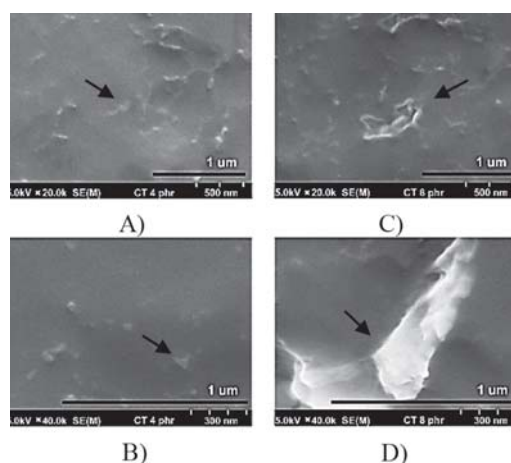


Fig. 2 A-B) FE-SEM micrograph of NRL/CT composites containing 4.0 phr of CT at magnification of 20k and 40k, respectively, and C- D) NRL/CT composites containing 8.0 phr of CT at magnification of 20k and 40k, respectively

3.2 Effect of CT content on NRL/CT latex properties

It could be seen from **Table 3** that the %TSC, pH and CN were not significantly changed with increasing CT content.

3.3 Effect of CT content on physical properties of NRL/CT composites

The effect of CT content on tensile strength of NRL/CT composites was shown in **Fig. 3**. It is widely accepted that tensile strength of NR vulcanizate depends on the degree of crystallization, crosslink density and also the presence of nanofillers. In this work, the tensile strength did not change significantly with increasing CT content up to 1 phr (the variation found was within the acceptable experimental error). However,

the tensile strength tended to decrease continuously at higher CT contents (>1 phr), probably due to the aggregation of CT particles at high contents as shown in **Fig. 2D**. Generally, the reinforcing nanofillers giving the enhancement of tensile strength should have a particle size of between 20 nm to 100 nm [24]. However, the CT particles used in this work had relatively large particle size which could act as a flaw in the rubber matrix leading to the reduction in tensile strength with increasing CT content.

Table 3 Effect of CT content on NRL/CT latex properties

CT content (phr)	% TSC	pH	CN
0.0	45.67	10.17	2
0.5	45.09	10.23	2
1.0	45.32	10.14	2
2.0	45.82	10.33	2
4.0	45.21	10.45	2
8.0	45.83	10.16	2

The elongation at break (**Fig. 4**) decreased with increasing CT content because the dispersion of CT filler became less homogenous (see **Fig 2D**). The CT reduced the molecular mobility of rubber chains and also resulted in a lower percentage of elongation at break and an increase in modulus [21].

The reduction in elongation at break was in concurrent with increasing stiffness of the NRL/CT composites. The overall modulus (**Fig. 5**) increased with increasing CT content. As the

modulus indicates the stiffness of a material, the results showed that the chitosan imparted a stiffening effect to the rubber which could be explained by the dilution effect. This is a common phenomenon in rubber, i.e., when any filler is added, the stiffness of the rubber is increased [21].

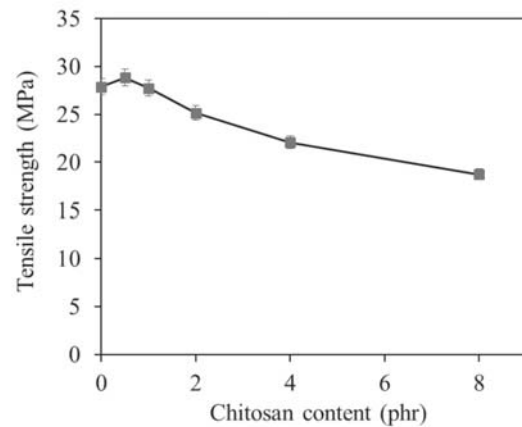


Fig. 3 Effect of CT content on tensile strength of NRL/CT composites

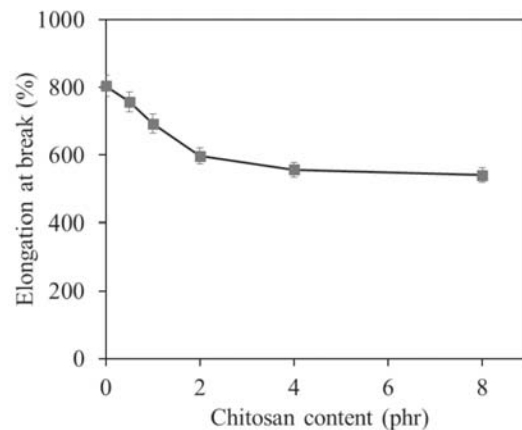


Fig. 4 Effect of CT content on elongation at break of NRL/CT composites

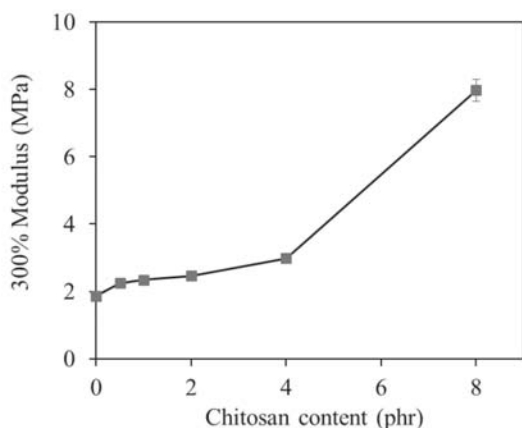


Fig. 5 Effect of CT content on 300% modulus of NRL/CT composites

Surprisingly, CT prepared in a dispersion form provided greater reinforcement, resulting in higher modulus, than other preparation methods including CT acid solution mixed with NRL [17-21], and CT dry mixed by a 2-roll milling process [22] as shown in **Table 4**.

Table 4 Effect of the different CT preparation methods on 300% modulus of NRL/CT composites

Methods	CT content (phr)	300% modulus (MPa)
Acid solution mixed [21]	10.0	3.0
Dry mixed [22]	10.0	2.7
Dispersion	8.0	8.1

According to Thai industrial standards (TIS 1056-1: 2013) for criteria of latex gloves, the tensile strength of the gloves should be higher than 7 MPa and the elongation at break should be at least

650%. The results revealed that the NRL/CT composites containing 0.5-1.0 phr of CT met the requirements of this standard. The CT content suitable for glove according to TIS 1056-1: 2013 was 0.5-1.0 phr. For other latex products that require high modulus such as tubing, pillow foam, rubber nipple and catheter, CT could be used at higher contents, e.g., from 2.0 to 4.0 phr.

3.4 Effect of CT content on inhibition of microbial growth of NRL/CT composites

The antibacterial activity of NRL/CT composite films, represented by the outer diameter of clear zone (free-living microorganism), was displayed in **Table 5** and **Fig. 6**. All NRL/CT composite films showed clear halo zones indicating that these rubber films inhibited the growth of *S. aureus* bacteria. The antimicrobial efficacy of the rubber was slightly enhanced in the presence of CT. It has been reported that the antimicrobial activity of chitosan results from its cationic nature [25].

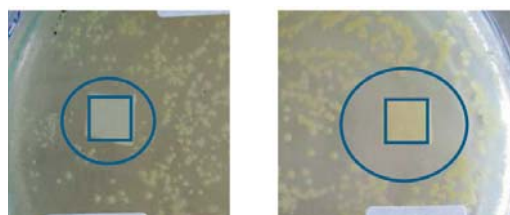


Fig. 6 The outer diameter of clear zone (A) non-CT rubber (B) 8 phr of CT incorporated rubber

Table 5 Effect of CT content on antibacterial activity of NRL/CT composite films

CT content (phr)	Clear zone diameters (cm)
0.0	1.63±0.12
0.5	1.84±0.23
1.0	1.89±0.17
2.0	1.91±0.15
4.0	1.95±0.11
8.0	1.98±0.16

4. Conclusion

Chitosan could be prepared in the form of dispersion. The latex properties was not significantly changed with increasing CT contents. With increasing CT content from 0.5-8.0 phr, 300 % modulus of the NRL/CT composite film was significantly increased whereas the opposite trend was found for tensile strength and elongation at break. The antimicrobial efficacy of the CT-incorporated rubber was slightly enhanced compared with that of the non-CT rubber. By comprehensive consideration, CT contents suitable for antibacterial gloves were 0.5-1.0 phr. For other latex products that required high modulus and antibacterial activity such as tubing, pillow foam, rubber nipple and catheter, CT could be used higher contents, i.e., 2.0 to 4.0 phr.

5. Acknowledgement

Financial support from the Thailand Research Fund, Thailand, is

acknowledged. The authors would like to express their appreciation to faculty of engineering and agro-industry, Maejo University for supporting the research facility.

6. References

- [1] D. J. Lamb, J. F. Anstey, C. M. Fellows, M. J. Monteiro, and R. G. Gilbert, "Modification of natural and artificial polymer colloids by "topology-controlled" emulsion polymerization," *Biomacromolecules*, vol. 2, pp. 518-525, 2001.
- [2] M. M. Rippel, L. T. Lee, C. A. P. Leite, and F. Galembeck, "Skim and cream natural rubber particles: Colloidal properties, coalescence and film formation," *Journal of Colloid and Interface Science*, vol. 268, pp. 330-340, 2003.
- [3] W. Pichayakorn, J. Suksaeree, P. Boonme, W. Taweepreda, and G. C. Ritthidej, "Preparation of deproteinized natural rubber latex and properties of films formed by itself and several adhesive polymer blends," *Industrial and Engineering Chemistry Research*, vol. 51, pp. 13393-13404, 2012.
- [4] E. Yip and P. Cacioli, "The manufacture of gloves from natural rubber latex," *Journal of Allergy and Clinical Immunology*, vol. 110, pp. S3-S14, 2002.
- [5] L. W. Hunt, P. Kelkar, C. E. Reed, and J. W. Yunginger, "Management

- of occupational allergy to natural rubber latex in a medical center: The importance of quantitative latex allergen measurement and objective follow-up,” *Journal of Allergy and Clinical Immunology*, vol. 110, pp. S96-S106, 2002.
- [6] Z. Peng, L. X. Kong, S.-D. Li, Y. Chen, and M. F. Huang, “Self-assembled natural rubber/silica nanocomposites: Its preparation and characterization,” *Composites Science and Technology*, vol. 67, pp. 3130-3139, 2007.
- [7] Y.-P. Wu, Y.-Q. Wang, H.-F. Zhang, Y.-Z. Wang, D.-S. Yu, L.-Q. Zhang, *et al.*, “Rubber-pristine clay nanocomposites prepared by co-coagulating rubber latex and clay aqueous suspension,” *Composites Science and Technology*, vol. 65, pp. 1195-1202, 2005.
- [8] H. H. Cai, S. D. Li, G. R. Tian, H. B. Wang, and J. H. Wang, “Reinforcement of natural rubber latex film by ultrafine calcium carbonate,” *Journal of Applied Polymer Science*, vol. 87, pp. 982-985, 2002.
- [9] Z. Peng, C. Feng, Y. Luo, Y. Li, and L. X. Kong, “Self-assembled natural rubber/multi-walled carbon nanotube composites using latex compounding techniques,” *Carbon*, vol. 48, pp. 4497-4503, 2010.
- [10] A. B. Suriani, M. D. Nurhafizah, A. Mohamed, I. Zainol, and A. K. Masrom, “A facile one-step method for graphene oxide/natural rubber latex nanocomposite production for supercapacitor applications,” *Materials Letters*, vol. 161, pp. 665-668, 2015.
- [11] P. Satraphan, A. Intasiri, V. Tangpasuthadol, and S. Kiatkamjornwong, “Effects of methyl methacrylate grafting and in situ silica particle formation on the morphology and mechanical properties of natural rubber composite films,” *Polymers for Advanced Technologies*, vol. 20, pp. 473-486, 2009.
- [12] H. Angellier, S. Molina-Boisseau, and A. Dufresne, “Mechanical properties of waxy maize starch nanocrystal reinforced natural rubber,” *Macromolecules*, vol. 38, pp. 9161-9170, 2005.
- [13] M. N. V. Ravi Kumar, “A review of chitin and chitosan applications,” *Reactive and Functional Polymers*, vol. 46, pp. 1-27, 2000.
- [14] A. Anitha, S. Sowmya, P. T. S. Kumar, S. Deepthi, K. P. Chennazhi, H. Ehrlich, *et al.*, “Chitin and chitosan in selected biomedical applications,” *Progress in Polymer Science*, vol. 39, pp. 1644-1667, 2014.
- [15] A. Domard, “A perspective on 30 years research on chitin and chitosan,” *Carbohydrate Polymers*, vol. 84, pp. 696-703, 2011.

- [16] S. C. Park, J. P. Nam, J. H. Kim, Y. M. Kim, J. W. Nah, and M. K. Jang, "Antimicrobial action of water-soluble β -chitosan against clinical multi-drug resistant bacteria," *International Journal of Molecular Sciences*, vol. 16, pp. 7995-8007, 2015.
- [17] J. Johns and V. Rao, "Mechanical properties and swelling behavior of cross-linked natural rubber/chitosan blends," *International Journal of Polymer Analysis and Characterization*, vol. 14, pp. 508-526, 2009.
- [18] J. Johns and V. Rao, "Mechanical properties of MA compatibilised NR/CS blends," *Fibers and Polymers*, vol. 10, pp. 761-767, 2010.
- [19] J. Johns and V. Rao, "Characterization of natural rubber latex/chitosan blends," *International Journal of Polymer Analysis and Characterization*, vol. 13, pp. 280-291, 2008.
- [20] J. Johns and V. Rao, "Thermal stability, morphology, and X-ray diffraction studies of dynamically vulcanized natural rubber/chitosan blends," *Journal of Materials Science*, vol. 44, pp. 4087-4094, 2009.
- [21] G. Raju and M. R. H. Mas Haris, "Preparation and characterization of acidified chitosan immobilized in epoxidized natural rubber," *Polymer Testing*, vol. 53, pp. 1-6, 2016.
- [22] H. Ismail, S. M. Shaari, and N. Othman, "The effect of chitosan loading on the curing characteristics, mechanical and morphological properties of chitosan-filled natural rubber (NR), epoxidised natural rubber (ENR) and styrene-butadiene rubber (SBR) compounds," *Polymer Testing*, vol. 30, pp. 784-790, 2011.
- [23] J. Jayadevan, R. Alex, and U. Gopalakrish-napanicker, "Chemically modified natural rubber latex - poly(vinyl alcohol) blend membranes for organic dye release," *Reactive and Functional Polymers*, vol. 112, pp. 22-32, 2017.
- [24] S. Boonrasri, P. Sae-Oui, S. Wirasate, and K. Suchiva, "Effects of bio-based oils on processing properties and cure characteristics of silica-filled natural rubber compounds," *Journal of Rubber Research*, vol. 20, pp. 1-19, 2017.
- [25] R. C. Goy, S. T. B. Morais, and O. B. G. Assis, "Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth," *Revista Brasileira de Farmacognosia*, vol. 26, pp. 122-127, 2016.