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Effect of shrimp shell chitosan loading on antimicrobial, absorption and morphological properties of natural rubber composites reinforced with silica−chitosan hybrid filler

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ABSTRACT
The natural rubber composites reinforced with hybrid filler between silica from rice husk and chitosan from shrimp shell were prepared by a latex solution method. The amount of shrimp shell chitosan was varied as 0, 3, 5, and 10phr with a constant amount of rice husk silica at 10phr. The natural rubber composites with hybrid filler were prepared before mixing with vulcanizing agents using conventional curing system. The antimicrobial, absorption, and morphological properties of the natural rubber composite films and cured composites were investigated by the Agar Diffusion Method, Water Absorption Test and Scanning Electron Microscopy (SEM), respectively. Using 5phr of chitosan shows the optimum properties for used as filler incorporated with silica in natural rubber composites for medical applications.
Keywords: *natural rubber composites; rice husk silica; shrimp shell chitosan; hybrid filler*; *antimicrobial activity; water absorption; morphological properties.*

1. INTRODUCTION
Chitosan is an amino-polysaccharide extracted from chitin which is the second biopolymer on earth. The sources of chitin are not only in the shells of shrimp and crab but also can be obtained in some insects and certain fungi. Chitosan is a biocompatible and biodegradable polymer. Moreover, it has antimicrobial activity [1-2]. A lot of research has focused on the utilize of chitosan as antimicrobial materials in biomedical applications [3-5]. Chitosan blended with natural rubber latex directly was shown to improve its thermal stability, mechanical, hydrophilic, and antimicrobial properties of natural rubber [6-7].

Rice husk is an agricultural waste from the rice milling process. Rice husk ash was used as a silica source that contained more than 60% of amorphous silica with high surface area, porosity and reactivity [8-11]. Silica plays an important role in some mechanical properties of natural rubber composites, not only to improve tensile strength, modulus, hardness, and wet traction but also reduce rolling resistance. To improve the ability of silica dispersion in natural rubber matrix, in-situ silica by sol-gel method was used for rubber composite preparation. Sodium silicate prepared from silica ash dissolution in sodium hydroxide was used as a precursor and it was become gel by acid solution [12-16].

Natural rubber or cis 1,4-polyisoprene is an elastomeric material that derived from the sap of rubber tree also known as natural rubber latex that contains the natural rubber hydrocarbon in a fine emulsion form in an aqueous serum [17]. It is an important economic crop in Thailand due to natural rubber products are the number one agricultural product with the highest export volume in the country. Natural rubber has the advantage namely low cost, renewable materials, and use in a variety of applications [18].

Many researchers have been prepared natural rubber composites reinforced with silica derived from rice husk or prepare natural rubber composites reinforced with chitin or chitosan that derived from shrimp shells to improve their properties [19-20]. In this work, natural rubber composites reinforced with both filler (silica-chitosan) were prepared and investigated their properties such as the antimicrobial properties, the absorption properties, and the morphological properties.

The aim of this work is to investigate the effect of shrimp shell chitosan on antimicrobial properties, absorption properties and morphological properties of natural rubber composites reinforced with silica-chitosan hybrid filler. Natural rubber composites were prepared by the latex solution method.

2. MATERIALS AND METHODS
2.1. Materials.
High ammonia natural rubber latex (HA Latex) was purchased from Viroonkit Industry Co., Ltd. (Thailand). It contains 60% Dry Rubber Content (DRC) and 0.7% ammonia. Shrimp shell chitosan (CS) with a degree of deacetylation of 65% and average molecular weight of $181x10^3$ Da were prepared in our laboratory. Sodium silicate solution was prepared by dissolving rice husk silica ash (86% of SiO_2) with 2.5% of sodium hydroxide. Other chemicals for rubber compounding such as stearic acid, zinc oxide, N-cyclohexyl-2-benzothiazole-2-sulfenamide (CBS) and sulphur were supported by Innovation Group (Thailand) Ltd.

2.2. Preparation of natural rubber composite films reinforced by silica-chitosan hybrid filler.
10phr of silica in the form of sodium silicate solution was added into NR latex that was diluted into 30% of DRC by distilled water. They were stirred for 12 hours until obtaining a homogeneous mixture. The dissolution of shrimp shell chitosan

with 2% acetic acid was used to prepare the chitosan solution. Then chitosan solution at various chitosan contents (0, 3, 5, and 10phr) was then added to the mixture of natural rubber latex and sodium silicate in order to precipitate silica in rubber latex. The pH of the mixture was adjusted to 7 by using 2% acetic acid. The mixtures were stirred for 6 hours and cast into Petri dishes before oven-dried at 60°C for 2 days to obtain composite films.

2.3. Preparation of vulcanized natural rubber composites.

The composite films were compounded by a two-roll mill machine. The ingredients for compounding were shown in Table 1. The compounding was started by mastication of NR composite film before adding stearic acid, ZnO, CBS and sulphur. After mixing 15 min, the compound was stored for 24 hours at room temperature. Curing time of the compound was determined by Moving Die Rheometer (MDR) at 150°C. The compounds were vulcanized in a hydraulic press at 150°C with a pressure of 150 kg/cm^2 with the curing time from MDR testing.

2.4. Characterization of natural rubber composite films.

2.4.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrometer was used to identify the functional groups of the composite films. The frequency range 4000 - 400 cm^{-1} at resolution of 4 cm^{-1}.

2.4.2 Antimicrobial Activity

The antimicrobial activity of the composite films was evaluated using the agar diffusion method. Escherichia coli (*E.coli*) bacteria was used for testing the antimicrobial activity. The composite films were placed on Mueller Hinton agar medium that had been seeded with 10^5cfu/ml (Colony Forming Units/mL) of the *E.coli*. The plates were incubated at 37°C for 24 hours. The antimicrobial properties of the composite films were observed from an inhibitory effect of microbial growth by measured the diameter of the inhibition zone around the composite films and cured composites.

2.4.3 Absorption Test

The cured composite samples were cut into 10x10 mm square sheet with thickness of 1 mm. Each sample was oven dried at 80°C and they were cooled in desiccator before weighing (W_1) then placed in Erlenmeyer flasks of distilled water for 2 days at a temperature of 37°C in the oven. After that, they were taken out from the oven and wiped with a tissue paper before weighing (W_2). The water absorption was calculated as follows:

$$\% \text{ Absorption} = \frac{W_2 - W_1}{W_1} \times 100 \quad \textbf{(Eq. 1)}$$

2.4.4 Scanning Electron Microscopy (SEM)

The surface morphology of the composite films was observed using SEM. The samples were cut into small pieces then were dried and coated with gold by a sputter coater before testing.

Table 1. Compounding formulations.

Sample	NR (phr)[a]	SiO_2 (phr)[a]	Chitosan (phr)[a]	Stearic acid(phr)[a]	ZnO (phr)[a]	CBS (phr)[a]	Sulphur (phr)[a]
NR	100	-	-	1	5	1.2	3
NR/$SiO_2$10	100	10	-	1	5	1.2	3
NR/$SiO_2$10-CS3	100	10	3	1	5	1.2	3
NR/$SiO_2$10-CS5	100	10	5	1	5	1.2	3
NR/$SiO_2$10-CS10	100	10	10	1	5	1.2	3

[a] part per hundred rubber

3. RESULTS

3.1. FTIR spectral analysis.

FTIR spectrum of NR and the composite films is shown in Figure 1.

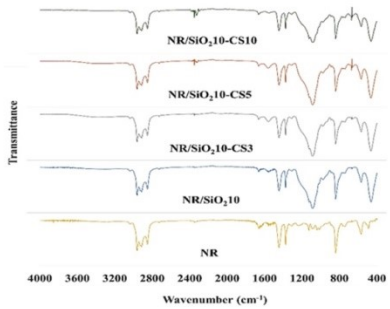

Figure 1. The FTIR spectrum of NR and the composite films

Basically, pure NR shows characteristic peak at 842 cm^{-1} represented =CH out of plane bending. Peak at 1375 cm^{-1}and 1432 cm^{-1}are characteristic of CH_2 deformation and at the region 2852-2925 cm^{-1} represented the CH_2 symmetric stretching vibrations [21-22]. All of NR composites filled SiO_2 show a strong peak at 464 and 1086 cm^{-1} that were attributed to the asymmetric bending vibration and the asymmetrical stretching vibration of Si-O-Si, respectively [23-24].

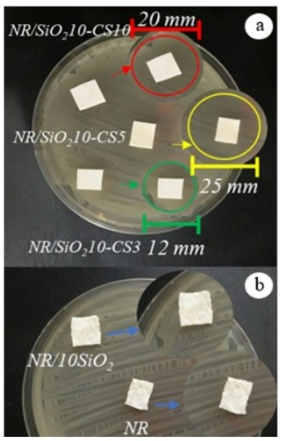

Figure 2. Effect of chitosan loading on antimicrobial activity of natural rubber composite films (a) with CS and (b) without CS.

The region between 908-1259 cm^{-1}of pure NR was intercepted by the asymmetrical stretching vibration of Si-O-Si in NR composites. NR composites filled chitosan, especially NR/$SiO_2$10-CS10 show a peak at 2350 cm^{-1} that is a characteristic of N-H stretching. The height of the peak increased with

increasing chitosan content. This may be due to the interaction between NR and chitosan or silanol groups of SiO_2 and chitosan [25]. Each peak confirmed the characteristic and presence of NR, SiO_2, and chitosan in the composites.

3.2. Antimicrobial properties.

The effect of chitosan loading on inhibition of microbial growth of the composite films and cured composites is shown in Table 2, Figure 2, and Figure 3.

The inhibition of microbial cells growth by chitosan was due to a positive charge of chitosan interferes with bacteria metabolism by stacking on the negative charge of bacterial surface [5].

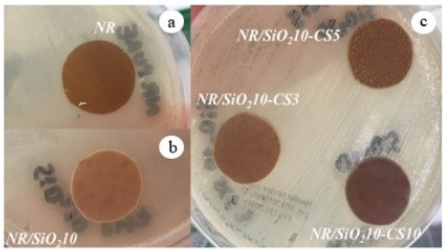

Figure 3. Effect of chitosan loading on antimicrobial activity of cured natural rubber composite (a)and(b) without CS and (c) with CS.

The antibacterial activity of the composite films and cured composites were represented by inhibition zone or clear zone around the samples. All of the composite films show the clear zone of *E. coli* inhibition and the highest diameter of the clear zone is NR/$SiO_2$10-CS5 following by NR/$SiO_2$10-CS10 and NR/$SiO_2$10-CS3. Although NR-$SiO_2$10-CS10 has the most of chitosan loading, its diameter of the clear zone was less than that of NR/$SiO_2$10-CS5. This may be due to the interaction of chitosan and other components which resulted in a decrease in its antimicrobial efficiency. For cured composites, no clear zone was observed. This may be due to the unactive positive charge of chitosan. However, the antimicrobial activities of chitosan depend on molecular weight (Mv) and the degree of deacetylation (DD). Chitosan with a higher degree of deacetylation tends to have higher antimicrobial activity due to an increase in positive charges [5].

Table 2. The diameter of inhibition zone of natural rubber and natural rubber composites.

	Composite Films			Cured Composites		
CS content (phr)	3	5	10	3	5	10
Clear Zone diameter (mm)	12	25	20	0	0	0

3.3. Absorption properties.

The water absorption behavior as a function of time of the cured composites was shown in Figure 4. The water absorbability of NR composite was increased with the addition of chitosan due to the hydrophilic nature of chitosan. However, water absorbability of NR/ $SiO_2$10-CS10 is less than NR/10SiO_2-CS3 and NR/ $SiO_2$10-CS5. This may be due to better interaction between NR and chitosan which shown by the FTIR.

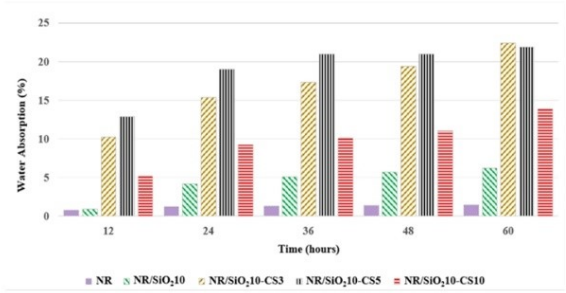

Figure 4. Graph of water absorption

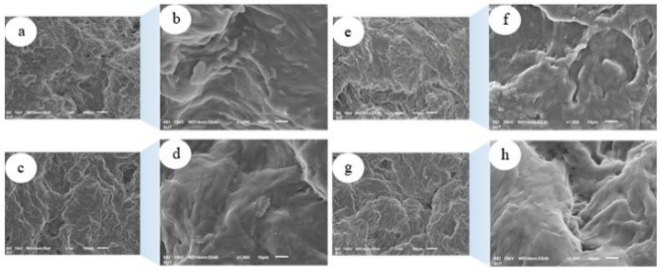

Figure 5. SEM micrographs showing the surface of natural rubber composites reinforced with silica and hybrid filler between silica and chitosan at 100X and 1000X magnification (a), (b) NR/ $SiO_2$10-CS3, (c), (d) NR/ $SiO_2$10-CS5, (e), (f) NR/ $SiO_2$10-CS10 and (g), (h) NR/ $SiO_2$10

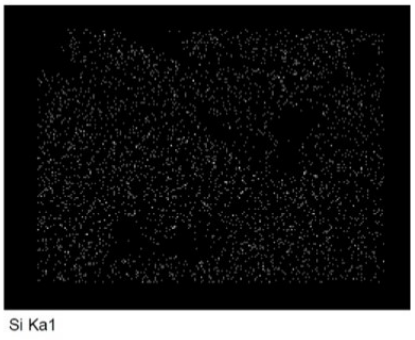

Figure 6. Energy dispersive X-ray (EDX) mapping analysis of silicon in NR/$SiO_2$10 composite.

3.4. Morphological properties.

SEM micrographs of the composite films are shown in Figure 5. All of the samples show the same surface morphology and no agglomeration of silica and incompatible zone in NR matrix. Si atoms are dispersed on the surface of the composites, as shown in the SEM-EDX mapping images in Figure 6.

4. CONCLUSIONS

Natural rubber composites reinforced with hybrid filler between rice husk silica and shrimp shell chitosan were successfully prepared by a latex solution method. All of NR composites that with the addition of shrimp shell chitosan show antimicrobial activity. The most efficient *E. coli* inhibition and the absorption properties suitable for use as wound dressing was obtained from NR composite with 5phr shrimp shell chitosan. Each NR composites show the same surface morphology. The silica agglomerate was not shown.

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