Study of Chitosan and Its Derivatives as Preservatives for Field Natural Rubber Latex

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ABSTRACT: In this study, the chitosan and its derivatives were tested for their preservative activities for field natural rubber (NR) latex. A series of chitosans with different molecular weights were obtained by nitrous acid depolymerization. The chemically modified chitosans, *N*-carboxymethyl chitosan (*N*CMCh), *N*-sulfated chitosan (*N*SCh), and *N*-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride (*N*HTACh), were prepared from high and low-molecular weight chitosans. Preservative activities for field NR latex of these chitosans were investigated based on the measurement of volatile fatty acids (VFA) number of the treated latex. The preservative activities of chitosan increased with decreasing molecular weights. The low-molecular weight *N*SCh and *N*HTACh exhibited good preservative activity for the latex. By the use of low-molecular weight *N*HTACh in combination with octylphenol poly (ethyleneglycolether) (Nonidet P40), the latex was successfully preserved for more than 1 month in the low-ammonia condition. The results showed an attractive feature to develop the preservative system, which was possible to reduce the concentrations of ammonia and carcinogenic nitrosamine in the NR latex. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 913–921, 2012

Key words: chitosan; natural rubber latex; preservative; volatile fatty acid number; surfactant

INTRODUCTION

Because microorganism causes spontaneous coagulation and putrefaction of natural rubber (NR) latex, after it leaves the tree, *Hevea brasiliensis*, preservation of the latex is necessary.[1] NR latex obtained as field latex usually preserved by the addition of ammonia either alone or combination with other bactericides. Ammonia is normally added at levels of 0.3–0.8% by weight of latex, depending on the required length of time preservation. Although ammonia possesses several satisfactory preservative properties, such as antibacterial activity, it also has a few disadvantages. One of disadvantages arises when the preserved latex containing ammonia is processed, because the emitted ammonia fumes pollute or adversely affect the environment. The emission of the ammonia fumes makes the working environment very unpleasant for the workers or other personnel and may indeed be injurious or hazardous to their health. Another problem arising from the emission of ammonia fumes relates to corrosion of factory buildings and roofs. Moreover, because ammonia is easily evaporated, the amount of ammonia in the latex is also hardly controlled.

When using ammonia in combination with other bactericides, for example, tetramethylthiuram disulphide (TMTD) and zinc oxide (ZnO), the dosage of ammonia added to preserve the latex can be reduced to 0.2–0.5%. But another problem with the use of TMTD is that TMTD is known to produce carcinogenic nitrosamine in the latex system. Additionally, TMTD can cause discoloration in latex products. The problem of using ZnO is that ZnO might destabilize latex, because the latex has a tendency to thicken when ZnO is present.

In the present study, as a part of our program to search for the new NR latex preservatives, we focus on chitosan and its derivatives. Chitosan, as one of the most abundant natural biopolymers obtained from agricultural waste such as shrimp or crab shells, including its unique polycationic nature, has a wide range of applications in various fields, such as textile, food, and cosmetic industry.[2] Furthermore, chitosan and its derivatives are of special interest in medical applications and are known to have some antibacterial and antifungal activities.[3] Liu et al.,[4]

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exploring the antibacterial activities of chitosans against *E. coli* by optical density method, found that the antibacterial activities of chitosans were influenced by its molecular weight, degree of deacetylation, concentration in solution, and pH of medium and found to be increased in the order of *N*,*O*-carboxymethyl chitosan, chitosan, and *O*-carboxymethyl chitosan. Seong et al.[5] used chito-oligosaccharide and *N*-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride (*N*HTACh) to treat cotton as antimicrobial agent. Jia et al.[6] found that the antibacterial activities of chitosan derivatives with quaternary ammonium salt in acetic medium are stronger than that in water. It was also found that the antibacterial activity of quaternized chitosan is stronger than that of chitosan. Muzzarelli et al.[7] indicated that methylpyrrolidinone chitosan, *O*-carboxymethyl chitosan, chitosan, and *N*-phosphonomethyl chitosan exerted effective fungistatic action against *S. parasitica*. Zhong et al.[8] synthesized acyl thiourea chitosan and studied its antimicrobial behavior with bacteria and pathogenic fungi. It was found that higher substitution resulted in stronger antifungal activity. Cao and Sun[9] prepared *N*-halalmine-based chitosan and found that it provided the total kill of 10^8–10^9 colony forming units (CFU/mL) of *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria) in 10 and 60 min, respectively. Moreover, the chlorinated chitosan effectively prevented the formation of bacterial biofilms. Chitosan, a cationic antibacterial agent may bind to the negatively charged bacterial surface to disturb the cell membrane and cause cell death due to leakage of intercellular components at low concentration and may additionally coat bacterial surface to prevent leakage of intercellular components as well as to impede mass transfer across the cell barrier at high concentration.[10] However, commercially available chitosan with high molecular weight commonly shows its biological activity only in acidic medium because of its poor solubility in water or basic media. Thus, it is difficult to use such chitosan to preserve NR latex, because the latex will lose its stability and be coagulated by acid. To overcome this difficulty, depolymerization and chemical modification of the commercially available chitosan to obtain chitosans with low-molecular weights and chitosan derivatives that are soluble in water were applied.

In this work, we prepared various types of chitosan and its three derivatives, *N*-carboxymethyl chitosan (*N*CMCh), *N*-sulfated chitosan (*N*SCh), and *N*HTACh, with different molecular weights and degrees of substitution (DS). Preservative activities for field NR latex of these chitosans were investigated based on measurement of volatile fatty acids (VFA) number of the treated latex. Further study to use chitosan derivatives in low ammonia system was also investigated.

EXPERIMENTAL

Materials

Commercially available chitosan (Chitosan-1) from shrimp shell supplied by AN Lab (Samutsakorn, Thailand) was used in depolymerizations and preparations of *N*CMCh-1, *N*SCh-1, and *N*HTACh-1. Its degree of deacetylation was determined to be 97.2% by solid-state ^{13}C nuclear magnetic resonance spectrometry (DPX 300, Bruker, Fallëndën, Switzerland). Field NR latex was obtained from Thai Rubber Latex Corp. (Thailand) PCL (Chonburi, Thailand). Octylphenol poly(ethyleneglycolether) (Nonidet P40) and other reagents were analytical grade purity and were used as received. Deionized water was used to prepare all solutions.

Depolymerization and characterization of chitosan

The nitrous acid (HNO_2) depolymerization of Chitosan-1 was carried out by addition of a fresh prepared aqueous solution of $NaNO_2$ to Chitosan-1 solution containing excess HCl for 20 min similar to the literature.[11] The calculation of desirable molecular weight of depolymerized chitosan is as follows:

$$M_2 = m_1/n(1 - f_d),$$

where M_2 is the molecular weight of depolymerized chitosan, m_1 is an initial mass of chitosan in solution, n is the moles of HNO_2, and f_d is the fraction of the initial HNO_2 that decomposes. Because 2,5-anhydro-D-mannose formed at the new reducing end is unstable as reported by Tømmeraas et al.,[12] a reduction to 2,5-anhydro-D-mannitol was carried out by using $NaBH_4$ for 15 min. Amount of $NaNO_2$ used for the depolymerization reaction in this study, molecular weights and molecular weight distributions of Chitosan-1 and depolymerized chitosans (Chitosan-2, Chitosan-3, Chitosan-4, Chitosan-5, Chitosan-6, and Chitosan-7) determined by gel permeation chromatography (600E fraction collector II, Water 2410, Milford, MA) with pullulan as standard, RI detector, one ultrahydrogel linear column and one guard column, 0.5*M* acetic acid/0.5*M* sodium acetate eluent, flow rate 0.6 cm^3/min, and operating temperature 25°C were presented in Table I.

Preparations and characterization of *N*CMCh, *N*SCh, and *N*Htach

N-carboxymethyl chitosan (*N*CMCh) was synthesized by a reaction of chitosan and glyoxilic acid according to the method reported by Sashiwa and Shigemasa.[13] Its DS was determined by pH titration[4] and then confirmed by ^1H-NMR, elemental analysis, and infrared spectroscopy.[14] *N*CMCh: ^1H-NMR

TABLE I
Depolymerization of Chitosan-1 by Nitrous Acid Depolymerization Method[a,b]

Samples	$NaNO_2$	M_w (kg/mol)	M_n (kg/mol)	M_w/M_n
Chitosan-1	–	532	184	2.89
Chitosan-2	0.050 g (0.72 mmol)	220	84.3	2.61
Chitosan-3	0.100 g (1.45 mmol)	110	44.4	2.48
Chitosan-4	0.260 g (3.77 mmol)	45.7	18.9	2.42
Chitosan-5	0.480 g (6.96 mmol)	23.0	10.4	2.21
Chitosan-6	0.840 g (12.2 mmol)	11.8	6.00	1.97
Chitosan-7	2.000 g (29.0 mmol)	4.17	2.38	1.75

[a] As reported by Allan and Peyron (1997).
[b] Chitosan-1 (16.0 g) was used as starting material.

(0.5*M* DCl/D_2O) δ = 1.98 (br s, —NH(CO)CH_3), 3.0–3.2 (br m, H-2 of *N*-carboxymethyl GlcN), 3.2–4.4 (br m, —NH—CH_2—, H-2 of GlcN and GlcNAc, H-3, H-4, H-5, and H-6), and 4.6–4.9 (br m, H-1).

N-sulfated chitosan (*N*SCh) was obtained by treating chitosan dispersed in dilute sodium carbonate solution with trimethylamine-sulfur trioxide complex. Its DS was determined by ^{1}H-NMR and elemental analysis as reported by Holme and Perlin.[15] *N*SCh: ^{1}H-NMR [D_2O contained Na_2CO_3 (pD = 9)] δ = 2.10 (br s, —NH(CO)CH_3), 2.74 (br s, H-2 of GlcN), 3.15 (br s, H-2 of *N*-sulfated GlcN), 3.2–4.4 (br m, H-2 of GlcNAc, H-3, H-4, H-5, and H-6), and 4.6–4.8 (br m, H-1).

*N*HTACh was synthesized by using a reaction of chitosan and glycidyltrimethylammonium chloride. Its DS was determined by ^{1}H-NMR and the measurement of chlorine content as reported by Seong et al.[5] *N*HTACh: ^{1}H-NMR (D_2O) δ = 1.98 (br s, —NH(CO)CH_3), 2.71 (br s, —NH—CH_2—), 2.87 [br s, H-2 of *N*-(2-hydroxy)propyl-3-trimethylammonium GlcN chloride], 3.15 (s, —NH(CH_3) $_3$Cl), 3.2–4.4 (br m, —CH_2— NH(CH_3) $_3$Cl, H-2 of GlcN and GlcNAc, H-3, H-4, H-5, and H-6), 4.60 (br s, —CH_2 —CH_2(OH)—CH_2—), and 4.85 (br s, H-1).

In the reactions, Chitosan-1 was used to prepare high-molecular weight chitosan derivatives, *N*CMCh-1, *N*SCh-1, and *N*HTACh-1. On the other hand, a depolymerized chitosan, Chitosan-6, was used to prepare low-molecular weight chitosan derivatives, *N*CMCh-6, *N*SCh-6, and *N*HTACh-6. Chemical structures of *N*CMCh, *N*SCh, and *N*HTACh were shown in Figure 1. In addition, *N*HTACh-2-5 with different molecular weights was synthesized from Chitosan-2-5 by using the same reaction as in the case of *N*HTACh-6. *N*HTACh-6LDS and *N*HTACh-6VLDS with different DSs were also prepared from Chitosan-6 by decreasing the amount of glycidyltrimethylammonium chloride used in the reaction. The structures of all the chitosan derivatives synthesized were confirmed by ^{1}H-NMR (DPX 300, Bruker, Fallëndën, Switzerland). DSs of the chitosan derivatives prepared in this work were summarized in Table II.

Field NR latex analysis

Total solid content (TSC) and dry rubber content (DRC) of field natural rubber (NR) latex were determined according to ASTM D 1076, which were 35.1% and 31.0%, respectively. The pH was tested to be 6.9 by using pH meter (IQ 240, IQ Scientific Instruments, San Diego, CA). Viscosity of the field latex was measured by viscometer (DV-II+, Brookfield, Middleboro, MA) operating at 80 rpm, 20°C. An average size of rubber particles in the latex was determined by photon correlation spectroscopy

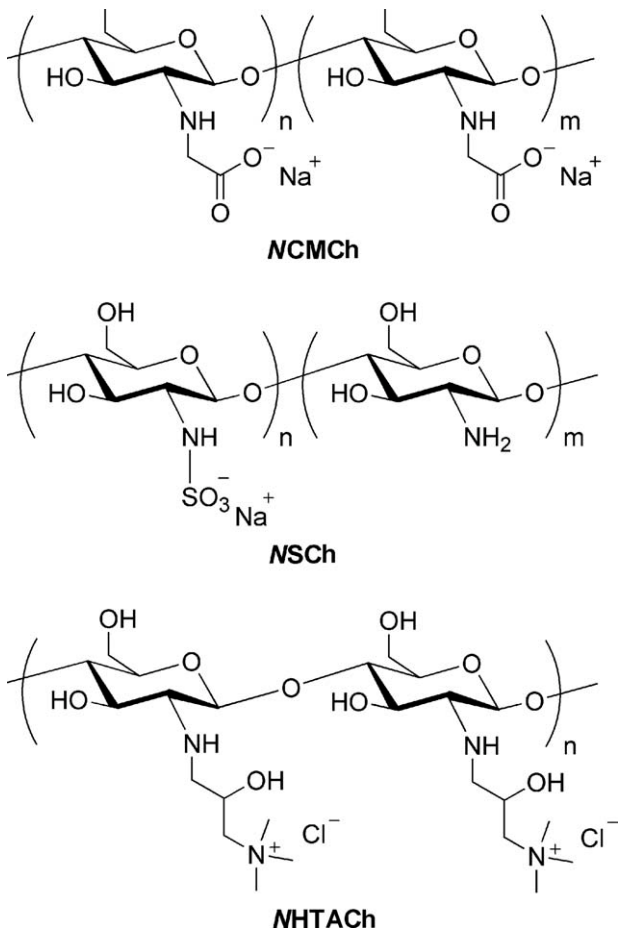

Figure 1 Structure of chitosan derivatives.

TABLE II
Starting Chitosans and Degrees of Substitution of Chitosan Derivatives

Samples	Starting chitosans	DSs[a]
*N*CMCh-1	Chitosan-1	1.02
*N*CMCh-6	Chitosan-6	1.05
*N*SCh-1	Chitosan-1	0.59
*N*SCh-6	Chitosan-6	0.46
*N*HTACh-1	Chitosan-1	0.99
*N*HTACh-2	Chitosan-2	1.02
*N*HTACh-3	Chitosan-3	1.01
*N*HTACh-4	Chitosan-4	0.98
*N*HTACh-5	Chitosan-5	1.03
*N*HTACh-6	Chitosan-6	1.03
*N*HTACh-6LDS	Chitosan-6	0.80
*N*HTACh-6VLDS	Chitosan-6	0.62

[a] Determined by ^{1}H-NMR.

(S4700, Malvern, Worcestershire, UK) using He-Ne laser source at a wavelength of 488 nm at 30°C. The scattering angle used was 90°.

VFA number, the number of grams of potassium hydroxide (KOH) required to neutralize the VFA in a latex sample containing 100 g of total solids, of the treated latex was determined periodically in accordance with the standard test (ASTM D 1076). Markham-Type Still apparatus was used for the test. The VFA number is measured by the quantity of barium hydroxide [$Ba(OH)_2$], which was required to neutralize the distilled-off short chain fatty acids. The calculation of the VFA number is as follows:

$$\text{VFA number} = (561 \times A \times N)/(W \times TS),$$

where A is an amount of $Ba(OH)_2$ solution in milliliters required for titration of the sample, N is the normality of $Ba(OH)_2$ solution, W is the mass of latex corresponding to 10 mL of acidified serum, and TS is a percentage of total solids in the latex.

Preservation of NR latex with chitosan

Chitosan with different molecular weights and chitosan derivatives was mixed with field natural rubber (NR) latex in various concentrations. The resulted mixtures were stored at room temperature for 90 days. Changes of VFA number and pH of latex during storage were investigated. Average sizes of rubber particles and viscosity of latex were also determined at 1 and 30 days.

Microbiological assay by viable cells count

A number of viable microorganisms in low-ammonia field NR latex treated with *N*HTACh-6 in various concentrations were determined by the pour-plating method as described in the literature.[16] Each sample was diluted with 0.9% NaCl in proper dilution. Then, 1 mL of the diluted samples was poured into the plate count agar medium, and the colonies were counted after 24-h incubation period at 37°C. The concentrations of microorganisms in the samples were determined as colony-forming unit in 1 mL: CFU/mL.

RESULTS AND DISCUSSION

Preservative activities of chitosans with different molecular weights

In the latex industry, four field NR latex properties, TSC, DRC, magnesium content, and the VFA number, of the latex were checked before the concentration process. Because bacterial degradation of latex constituents causes the formation of short-chain VFA, the VFA number is the most important measure of the level of deterioration and stability of the latex. Thus, the VFA number of the treated latex was used to indicate the preservative activities of the chitosans with different molecular weights and chitosan derivatives.

In the investigation of the dependence of the preservative activity on molecular weight of chitosan, seven types of chitosan with molecular weights ranging from 4.17 to 532 kg/mol (Chitosan-1–7) were studied. It was found that Chitosan-1–4 were not soluble in the water and an addition of Chitosan-5, a water-soluble chitosan with the molecular weight of 23.0 kg/mol, 10 g to the latex 1 L made the latex coagulated within 7 h. Because proteins absorbed on surfaces of rubber particles carry negative charges that prevent aggregation of the rubber particles, this chitosan, which is cationic polymers, might reduce or neutralize the surface charge and make the latex coagulated. On the other hand, the lower molecular weight Chitosan-6 and Chitosan-7, which are considered being worse bridging agents, could stabilize the latex although they caused the viscosity of the latex to increase.

Figure 2 shows the VFA number versus storage time of the latex treated with and without Chitosan-6 and Chitosan-7. It can be seen that Chitosan-6 was almost ineffective in suppressing increases of VFA. The lower molecular weight Chitosan-7 was noticeably more effective. Thus, the preservative properties of chitosan appeared to be dependent on its molecular weight, probably due to greater solubility of the lower molecular weight chitosan in the latex.

From this result, in the next section, however, Chitosan-6 was chosen here to be the starting material for the preparation of chitosan derivatives instead of Chitosan-7 to clearly investigate differences in the preservation activities of the modified and unmodified chitosans.

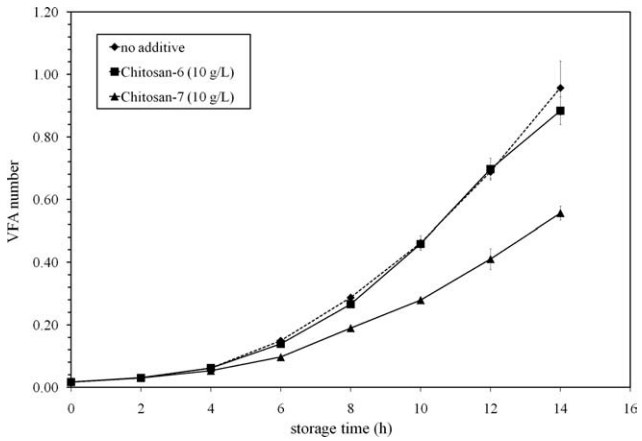

Figure 2 VFA number versus storage time of field NR latex treated by Chitosan-6, Chitosan-7, and latex without chitosan.

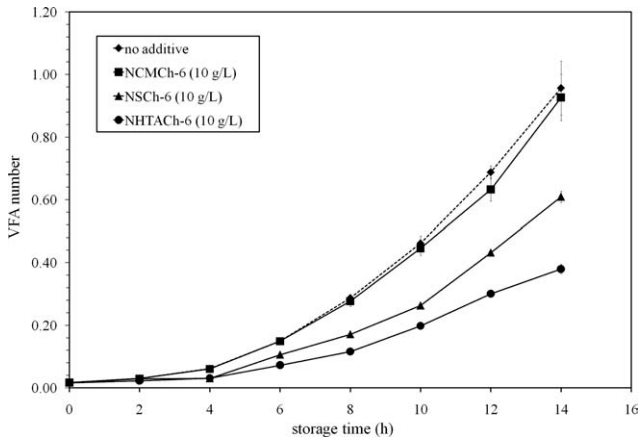

Figure 3 VFA number versus storage time of field NR latex treated by *N*CMCh-6, *N*SCh-6, *N*HTACh-6, and latex without chitosan derivatives.

Preservative activities of chitosan derivatives

In addition to chitosans with different molecular weights, in this section, six chitosan derivatives, *N*CMCh-1, *N*SCh-1, *N*HTACh-1, *N*CMCh-6, *N*SCh-6, and *N*HTACh-6 were studied. We first attempted to use the high-molecular weight chitosan derivatives (*N*CMCh-1, *N*SCh-1, and *N*HTACh-1) prepared from Chitosan-1, the commercially available chitosan, as the preservatives. Unfortunately, they could not stabilize the field NR latex. Similar to Chitosan-5, the addition of *N*HTACh-1, the high-molecular weight cationic polymer, 10 g/L made the latex coagulated within 7 h. On the other hand, in both cases of the high-molecular weight anionic chitosan derivatives, *N*CMCh-1 and *N*SCh-1, although no coagulation was observed as expected, it was found that after letting the latex stand for a while, the latex separated into two layers, cream and serum phases appeared similar to the cases of water-soluble hydrocolloids as mentioned in the literature.[1] Thus, the high-molecular weight chitosan derivatives were not suitable in using as the preservative of the field NR latex.

In contrast to *N*CMCh-1, *N*SCh-1, and *N*HTACh-1, no coagulation or creaming occurred in the studied period in the cases of *N*CMCh-6, *N*SCh-6, and *N*HTACh-6, which are the lower molecular weight chitosan derivatives prepared from chitosan-6. Figure 3 shows the VFA number versus the storage time of the field NR latex treated by these chitosan derivatives.

At first, the preservative activities of *N*CMCh-6 and *N*SCh-6, which are chitosan substituted with anionic functional groups, were investigated. Generally, to improve the water solubility, the introductions of anionic functional groups are effective methods. However, the antibacterial activities of modified chitosans were found to decrease with increasing degree of substitution (DS) of the amino group.[4,17] In this study, although the effect of the kind of the anionic functional groups introduced was not clear at the present time, the similar trends were observed. *N*CMCh-6 was not effective in preserving the NR latex. On the other hand, the VFA number of the latex treated with *N*SCh-6 that contained higher amino group content (see Table II) was much lower than that of the untreated latex. Figure 4 shows the VFA number versus the storage time of the field NR latex treated by various concentrations of *N*SCh-6. The VFA number decreased with increasing of the concentration of *N*SCh-6 in the latex. The VFA number of the latex was decreased from 0.817 to 0.501 by treating the latex with *N*SCh-6 15 g/L instead of *N*SCh-6 5 g/L for 14-h storage.

Not only *N*SCh-6, but *N*HTACh-6, the cationic chitosan derivative contained quaternary ammonium groups, was also effective in lowering the VFA number of the stored field NR latex, suggesting that the

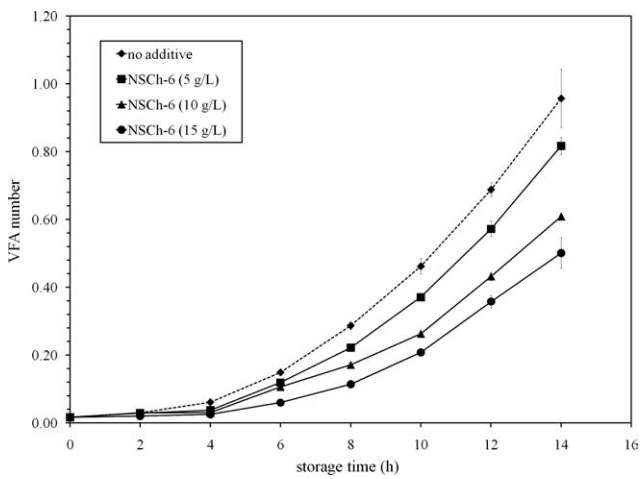

Figure 4 VFA number versus storage time of field NR latex treated by various concentrations of *N*SCh-6 and latex without *N*SCh-6.

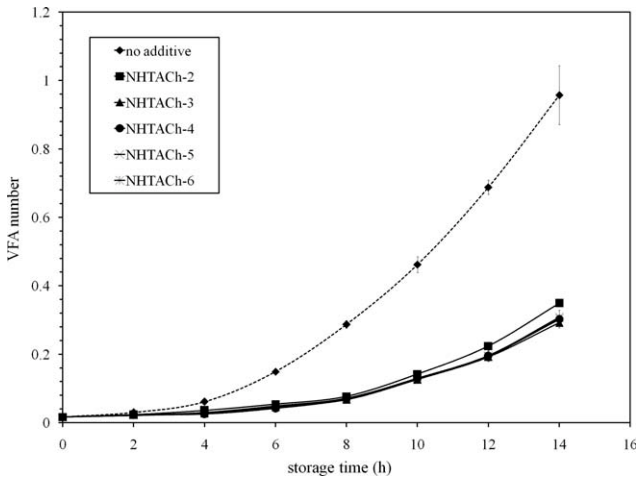

Figure 5 VFA number versus storage time of field NR latex treated by various molecular weights of *N*HTACh and latex without *N*HTACh.

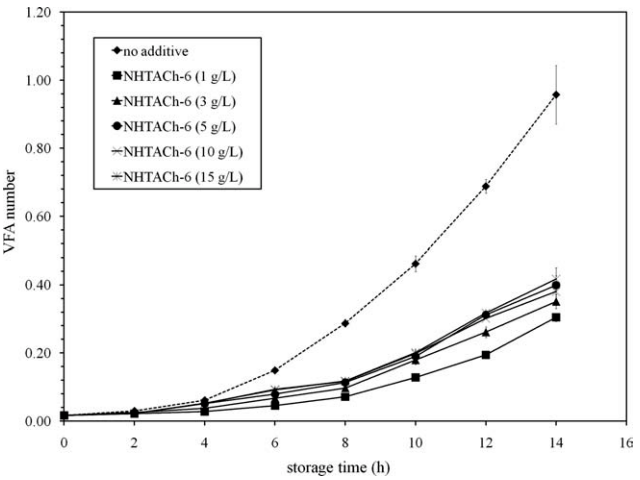

Figure 7 VFA number versus storage time of field NR latex treated by various concentrations of *N*HTACh-6 and latex without *N*HTACh-6.

quaternary ammonium groups introduced could enhance the preservative activity of chitosan. This agrees with reports in the literature.[5]

With the most effective activity to preserve the field NR latex, the effects of molecular weight and DS of *N*HTACh were further studied, and the results are shown in Figures 5 and 6, respectively. In contrast to the failure to use *N*HTACh-1 as a latex preservative, it was obviously found that *N*HTACh-2-5 prepared from Chitosan-2-5 with the molecular weights of 220–23.0 kg/mol also could slow the increasing of the VFA number similar to the case of *N*HTACh-6 and showed almost no difference on the preservative activity. Furthermore, as mentioned earlier that the quaternary ammonium groups could enhance the preservative activity of chitosan, Figure 6 confirmed this function of the trimethylammonium chloride group in *N*HTACh. The increase in DS of the trimethylammonium chloride group led to the decrease of VFA number of the latex, which implied to higher preservative activity.

Although *N*HTACh seemed to be the chitosan derivative that could be effectively applied to use as preservative for the field NR latex, it has been however observed that the VFA number decreased with decreasing of the concentration of *N*HTACh in the latex. As shown in Figure 7, after 14-h storage, the VFA number of the latex preserved with *N*HTACh-6 15 g/L and 1 g/L were 0.417 and 0.305, respectively. This result might arise from the increase in viscosity of the latex with increasing the concentration of *N*HTACh-6. The high viscosity might disturb solubility or dispersion of *N*HTACh-6 in the latex. To confirm this behavior, a commercially available nonionic surfactant, octylphenol-poly(ethyleneglycolether) (Nonidet P40), was added to the latex prior

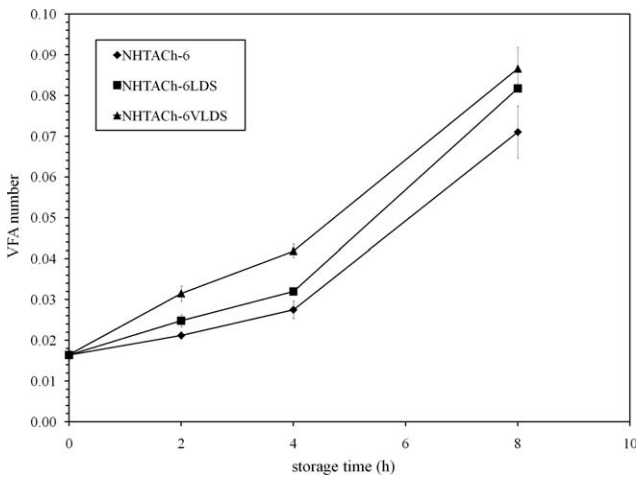

Figure 6 VFA number versus storage time of field NR latex treated by various degree of substitutions of *N*HTACh and latex without *N*HTACh.

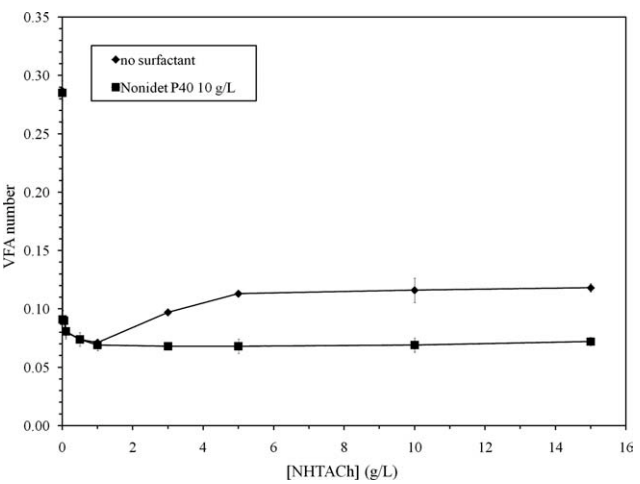

Figure 8 VFA number versus *N*HTACh-6 concentration of field NR latex with and without Nonidet P40 after 8-h storage.

TABLE III
VFA Number and pH of Field NR Latex Treated with *N*HTACh-L in Low-Ammonia Systems

Samples	VFA number (pH)					
	1 day	4 days	8 days	18 days	30 days	90 days
0.16 wt % ammonia latex	0.23 (9.9)	Coagulated				
0.30 wt % ammonia latex	0.018 (10.0)	0.025 (10.0)	0.15 (10.0)	>0.80 (9.6)	>0.80 (9.3)	>0.80 (8.5)
0.70 wt % ammonia latex	0.013 (10.5)	0.017 (10.5)	0.025 (10.6)	0.030 (10.6)	0.034 (10.4)	0.236 (10.3)
0.30 wt % ammonia latex + Nonidet P40 3 g/L	0.017 (10.0)	0.026 (10.1)	0.14 (10.0)	>0.80 (9.5)	>0.80 (9.2)	>0.80 (8.3)
0.16 wt % ammonia latex + *N*HTACh-6 1 g/L	0.034 (9.8)	Coagulated				
0.30 wt % ammonia latex + *N*HTACh-6 1 g/L	0.018 (10.0)	0.027 (10.0)	0.035 (10.0)	0.039 (10.0)	0.044 (9.7)	0.134 (9.5)
0.30 wt % ammonia latex + Nonidet P40 1 g/L + *N*HTACh-6 1 g/L	0.014 (10.0)	0.019 (10.0)	0.029 (10.0)	0.032 (10.0)	0.040 (9.9)	0.133 (9.5)
0.30 wt % ammonia latex + Nonidet P40 3 g/L + *N*HTACh-6 1 g/L	0.013 (10.0)	0.018 (10.0)	0.026 (10.0)	0.031 (10.0)	0.038 (9.9)	0.125 (9.5)

treating with *N*HTACh-6 to increase the stability of the latex and maintain its viscosity. Because, the use of anionic surfactants might interfere with the activity of the cationic chitosan, the nonionic surfactant was chosen in this study. As expected, with the addition of Nonidet P40, the preservative activity of *N*HTACh-6 was found to be improved, especially in the case containing high concentration of the chitosan. Figure 8 illustrated this result. By adding Nonidet P40 10 g/L, the VFA number of the latex contained *N*HTACh-6 15 g/L decreased from 0.118 to 0.072 after 8-h storage.

Preservative activities of *N*Htach-6 in low-ammonia system

Although the ammonia/TMTD/ZnO system is a well-known low-ammonia preservation system for NR latex industry in nowadays, TMTD possibly generates nitrosamines, which are carcinogenic substances. In the preceding section, we confirmed the preservative activity of *N*HTACh-6 from the results in the nonammonia system. *N*HTACh-6 was the most effective chitosan derivative in the study. The VFA number of the field NR latex treated with *N*HTACh-6 was low even by using at very low concentration. In this section, it is therefore of interest to investigate the preservative activity of ammonia in combination with *N*HTACh-6 instead of TMTD/ZnO.

The results from Table III showed that the preservation of field NR latex was depended on the concentration of ammonia used as mentioned in the literature.[1] Although pH value of field NR latex was rather constant, VFA number of field NR latex treated with 0.16 wt % ammonia was 0.23 on the first day and, then, the latex coagulated within 4 days. Whereas VFA number of field NR latex treated with 0.30 and 0.70 wt % ammonia could be kept to

TABLE IV
Average Sizes of Rubber Particles and Viscosity of Field NR Latex Treated with *N*HTACh-L in Low-Ammonia Systems

Samples	Average size of rubber particles[a] (nm)		Viscosity[b] (cP)	
	1 day	30 days	1 day	30 days
0.16 wt % ammonia latex	ND[c]	ND[c]	25.6	ND[c]
0.30 wt % ammonia latex	298.2	302.4	21.9	23.0
0.70 wt % ammonia latex	285.6	282.3	20.5	20.3
0.30 wt % ammonia latex + Nonidet P40 3 g/L	280.1	283.1	20.7	20.7
0.16 wt % ammonia latex + *N*HTACh-6 1 g/L	ND[c]	ND[c]	213	ND[c]
0.30 wt % ammonia latex + *N*HTACh-6 1 g/L	1070	1108	159	163
0.30 wt % ammonia latex + Nonidet P40 1 g/L + *N*HTACh-6 1 g/L	528.2	532.3	62.0	64.2
0.30 wt % ammonia latex + Nonidet P40 3 g/L + *N*HTACh-6 1 g/L	278.3	280.1	21.2	20.9

[a] Determined by PCS.
[b] Determined by viscometer operating at 80 rpm.
[c] ND, no data.

TABLE V
Concentration of Bacteria in Field NR Latex After 2 Days Storage

Samples	Concentration of bacteria (CFU/mL)
0.30 wt % ammonia latex	$3.13 \times 10^6 \pm 0.35 \times 10^6$
0.30 wt % ammonia latex + *N*HTACh-6 0.01 g/L	$1.23 \times 10^6 \pm 0.05 \times 10^6$
0.30 wt % ammonia latex + *N*HTACh-6 0.05 g/L	$1.09 \times 10^6 \pm 0.14 \times 10^6$
0.30 wt % ammonia latex + *N*HTACh-6 0.1 g/L	$5.10 \times 10^5 \pm 0.07 \times 10^5$
0.30 wt % ammonia latex + *N*HTACh-6 0.5 g/L	$8.00 \times 10^4 \pm 0.14 \times 10^4$
0.30 wt % ammonia latex + *N*HTACh-6 1 g/L	$2.50 \times 10^4 \pm 0.01 \times 10^4$
0.30 wt % ammonia latex + Nonidet P40 3 g/L + *N*HTACh-6 1 g/L	$5.00 \times 10^3 \pm 0.06 \times 10^3$

the value less than 0.08 (the acceptable limit of VFA number for the concentrated latex industry) for 8 days and 1 month, respectively.

When *N*HTACh-6 was added to the ammonia preserved as a secondary preservative, it was found that the VFA number of field NR latex containing 0.30 wt % ammonia and treating with *N*HTACh-6 1 g/L was slowly increased with storage times when compared with untreated sample. VFA number was decreased from more than 0.15 that was over the acceptable limit of VFA number for the concentrated latex industry (0.08) to 0.035 for 8 days and remained at 0.044 for 1 month. This indicated that *N*HTACh-6 was very effective in suppressing increase of VFA in the latex. Although the field NR latex containing 0.16 wt % ammonia and treating with *N*HTACh-L 1 g/L coagulated within 4 days, similar to the untreated sample, its VFA number on the first day was much lower than that of the untreated one. This result might be used to support that *N*HTACh-6 could act as a secondary preservative, which effectively maintained VFA number of the ammonia preserved field NR latex during storage.

However, it was seen in Table IV that size of rubber particles and viscosity of the ammonia preserved field NR latex were much increased when the latex was treated with *N*HTACh-6. This could be explained that *N*HTACh-6 containing positively charged quarternary ammonium groups caused a lowering of the stability of the negatively charged NR particles. To overcome this problem, here, Nonidet P40 was applied. Although Nonidet P40 itself could not suppress the increase of VFA in the latex as can be seen in Table III, the addition of the nonionic surfactant was found to be an effective way to overcome the stability problem of the latex when using *N*HTACh-6 as the secondary preservative. By adding Nonidet P40 3 g/L to the latex prior treating with *N*HTACh-6 1 g/L, the VFA number of the latex was very low. Moreover, the average size of rubber particles and the viscosity of the latex was also found to be closed with those of the original latex without *N*HTACh-6.

Finally, to directly determine the antimicrobial activity of *N*HTACh-6 in 0.30 wt % ammonia latex, the numbers of viable organisms in the latex treated by various concentrations of *N*HTACh-6 and latex without *N*HTACh-6 after 2 days storage were investigated. The results are summarized in Table V. The concentrations of bacteria in samples decreased with increasing the concentration of *N*HTACh-6. Again, it was found that the presence of Nonidet P40 not only homogenized the system, but also improved bactericidal efficiency. The 0.30 wt % ammonia latex-containing Nonidet P40 3 g/L and *N*HTACh-6 1 g/L revealed the most antibacterial efficiency. It contained viable bacteria 5.00×10^3 CFU/mL, only 0.16% of the concentration of bacteria in the sample without *N*HTACh-6. It can be seen that the resulting low-ammonia field NR latex treated by Nonidet P40 and *N*HTACh-6 possessed low number of bacteria and low-VFA number. The average size of rubber particles and the viscosity of the latex almost unchanged from the starting latex. Thus, all data show the successful preservation of the field NR latex by using the developed system.

CONCLUSIONS

The present study showed that the solubility and the functional group of chitosan and chitosan derivatives seemed to be important factors affecting the preservative activities. The low-molecular weight chitosan, *N*SCh, and *N*HTACh exhibited preservative activity for the field NR latex, especially in the case of the low-molecular weight *N*HTACh that contains quaternary ammonium salt groups. With the use of the low-molecular weight *N*HTACh in combination with a nonionic surfactant, Nonidet P40, the latex was successfully preserved for more than 1 month in the low-ammonia condition. An attractive feature of the developed system showed the possibility to reduce the concentration of ammonia in the latex. In addition, this system could preserve the latex without potential formers of carcinogenic nitrosamines, TMTD.

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