Research Article

Deproteinization of Nonammonia and Ammonia Natural Rubber Latexes by Ethylenediaminetetraacetic Acid

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Deproteinized natural rubber (DPNR) was made from sodium hydroxymethylglycinate latex (SH-latex) and ammonia latex (NH₃-latex) by mixing with different forms of ethylenediaminetetraacetic acid (EDTA) in the presence of sodium dodecyl sulfate (SDS). The mixtures were stirred at room temperature followed by centrifugation to separate the denatured proteins. The optimized reaction contained 0.01 wt% ethylenediaminetetraacetic acid tetrasodium (EDTA-4Na) with 1% SDS. The nitrogen contents of the DPNR from SH-latex and NH₃-latex were reduced to 0.005 wt% and 0.008 wt%, respectively, compared to 0.551 wt% in the starting SH-latex and 0.587 wt% in the NH₃-latex. SDS-PAGE analysis and FT-IR spectroscopy showed decomposition of latex proteins to peptides of smaller molecular weight. Physical properties of the DPNR rubber were studied. The novel EDTA-4Na treatment is considered an effective deproteinization method with potential application on both ammonia and nonammonia preservative systems.

1. Introduction

Natural rubber (NR) field latex (*Hevea brasiliensis*) contains 30–40% rubber by weight dispersed as rubber latex particles in water with some minor nonrubber constituents such as proteins, lipids, carbohydrates, sugars, and metal ions. The rubber particles of fresh rubber latex contain *cis*-1,4-polyisoprene surrounded by proteins and lipids on the outer surface. The protein component of the natural rubber latex particles is believed to be exclusively associated with the particle surface as an adsorbed layer [1]. These proteins present on the surface of NR particles in the latex state may sometimes bring about latex allergy mediated by type I immunoglobulin E [2, 3]. The potential protein allergy risk in NR products for sensitized users has resulted in an increasing demand on deproteinized NR latex, particularly for medical devices and hypoallergic products.

Different chemical and enzymatic methods have been reported for deproteinization of NR. NR was effectively

deproteinized in the latex stage by enzymatic degradation which removed proteins present on the surface of the rubber particle [4, 5]. This resulted in marked decrease in the nitrogen content of NR to less than 0.02%, which was approximately 5% of the starting material [6–8]. However, the enzymatic process requires long incubation time (usually more than 24 h) while the remaining proteins, peptides, or amino acids may result in intraoperative anaphylactic reactions of hypersensitive patients of allergy [3, 9]. NR treatment with urea and saponification led to reduction in the total nitrogen content to lower than 0.02 wt% within 1 hour under suitable conditions [6].

The conventional ammonia system for latex preservation using chemicals such as TMTD (Tetra methyl thiuram disulfide) and ZnO as secondary preservatives that are highly toxic can potentially cause allergy. This leads to an increasing trend on using nonammonia preservatives for fresh latex. Development of an efficient method for deproteinization of NR preserved by nonammonia system is thus of great interest.

SH preservative has no strong smell, is free of nitrosamine, and is nontoxic to cells. The pH of SH-latex can be adjustable to 8–11 which is considered advantageous for subsequent application.

In this present work, the use of ethylenediaminetetraacetic acid tetrasodium (EDTA-4Na) in conjunction with surfactant on deproteinization of nonammonia and ammonia latices was studied. This can potentially cause chelation of the metal ions coordinate with NR proteins, resulting in denaturation and removal of the proteins from NR surface. The deproteinized rubber was characterized for physical properties. This work demonstrates the novel EDTA treatment as an effective alternative for NR deproteinization for preparation of deproteinized latex for allergenic free applications.

2. Experimental

2.1. Materials. Fresh natural rubber (FNR) latex was collected by tapping from mature *Hevea* rubber trees of Thai Rubber Latex Co., Ltd., plantation in Chonburi province, Thailand. Sodium hydroxymethylglycinate (SH) was provided from ISP Chemical (Columbus, Ohio, USA). Ethylenediaminetetraacetic acid, Coomassie Brilliant Blue R-250, sodium dodecyl sulfate, lauric acid, potassium hydroxide, and ammonium hydroxide were analytical grade and supplied by Sigma-Aldrich. Ammonium persulfate, N,N,N',N'-tetramethylethylenediamine (TEMED), and acrylamide were procured from Biorad Laboratories, Inc. (Hercules, CA).

2.2. Methods

2.2.1. Preservation of NR Latex. The fresh natural rubber (FNR) latex was divided into two fractions. The first fraction was suddenly preserved by the addition of 0.4 wt% cosmetic grade preservative, sodium hydroxymethylglycinate (SH) [10], and adjusted the pH to 10 using potassium hydroxide (KOH) and used as the nonammonia latex sample. The second fraction was added with 0.4 wt% ammonium hydroxide and used as the ammonia latex sample.

2.2.2. NR Deproteinization. The DPNR latex was prepared by treating FNR latex with ethylenediaminetetraacetic acid sodium (EDTA-Na), ethylenediaminetetraacetic acid disodium (EDTA-2Na), ethylenediaminetetraacetic acid trisodium (EDTA-3Na), and ethylenediaminetetraacetic acid tetrasodium (EDTA-4Na) at various concentrations (0.05, 0.10, 0.20 wt%) in the presence of 1 wt% sodium dodecyl sulfate (SDS) at room temperature for 1 h with continuous stirring. Then, the treated latex was centrifuged at 10,000 rpm for 50 min. The cream fraction was redispersed in 1 wt% SDS solution to make 30% dry rubber content (DRC) latex and washed twice by centrifugation. The obtained cream fraction was redispersed in distilled water to make DPNR of 30% dry rubber content (DRC) latex. The samples of untreated and deproteinized latices were air-dried. The DPNR latex was coagulated with methanol for characterization of physical properties. The effects of different EDTA salt complexes were studied at 0.10 wt% of the chelating agent using the

FIGURE 1: Schematic representation of deproteinization procedure.

method described above. The deproteinization procedure is presented in Figure 1.

2.2.3. Analytical Methods. Protein profiles of DPNR were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the standard procedure [11] with 4% stacking gels and 15% separating gels at a constant current of 15 mA for 1 h. The molecular weight reference marker was run simultaneously in all experiments. The protein bands were visualized by staining Coomassie Blue R-250.

The total nitrogen content of the NR samples was measured by using the Kjeldahl method according to the Rubber Research Institute of Malaysia (RRIM) test method [12]. Functional groups of NR samples were characterized by Fourier transform infrared (FT-IR) spectroscopy using a Perkin Elmer Spectrum Spotlight 300 spectrometer (Perkin Elmer, USA). Physical properties of DPNR rubber were determined in accordance with SMR Bulletin No. 7-1992 for dirt content, volatile matter content, ash content, initial Wallace plasticity (P_0), and plasticity retention index (PRI) and Mooney viscosity.

3. Results and Discussion

3.1. Effects of EDTA-4Na Concentration. In order to determine the optimal dosage of EDTA-4Na on deproteinization, the nonammonia and ammonia latices were incubated with EDTA-4Na at various concentrations (0.05, 0.10, and 0.20 wt%) in the presence of 1 wt% SDS at room temperature for 1 h. Separation and washing of the cream fraction in the presence of 1 wt% SDS were later carried out. The final cream

TABLE 1: Nitrogen content of FNR and deproteinized FNR (DPNR) by using EDTA-4Na at various concentrations.

Samples	Preservative	pH	EDTA-4Na (wt%)	Nitrogen content (wt%)
FNR	—	6.5	—	0.638
FR-NH ₃	Ammonia	10	—	0.587
FR-SH	SH	10	—	0.551
FR-NH ₃	Ammonia	10	0.05	0.043
FR-NH ₃	Ammonia	10	0.10	0.009
FR-NH ₃	Ammonia	10	0.20	0.008
FR-SH	SH	10	0.05	0.012
FR-SH	SH	10	0.10	0.005
FR-SH	SH	10	0.20	0.005

TABLE 2: Magnesium ion content of FNRL and FNRL deproteinized by using EDTA-4Na.

Samples	Preservatives	EDTA-4Na (wt%)	Mg ²⁺ content (ppm)
FNRL	—	—	657
FR-NH ₃	Ammonia	—	294
FR-SH	SH	—	635
FR-NH ₃ DPNR	Ammonia	0.05	152
FR-NH ₃ DPNR	Ammonia	0.10	123
FR-NH ₃ DPNR	Ammonia	0.20	89
FR-SH DPNR	SH	0.05	417
FR-SH DPNR	SH	0.10	341
FR-SH DPNR	SH	0.20	289

fraction was redispersed in distilled water to make DPNR latex with 30% dry rubber content (DRC).

The total nitrogen contents of the fresh natural rubber (FNR) and deproteinized natural rubber (DPNR) is shown in Table 1. The nitrogen content of the air-dried FNR film was 0.638%. Increasing dosage of EDTA-4Na led to a decreasing trend of nitrogen content in the deproteinized NR preserved by both methods. Treatment of FR-NH₃ with 0.20 wt% EDTA-4Na led to a remarkable decrease of the nitrogen content from 0.587 wt% to 0.008 wt%. Moreover, the total nitrogen content of FR-SH DPNR was found to be the lowest when 0.10 wt% EDTA-4Na was used which resulted in the final nitrogen content of 0.005 wt% compared with 0.551 wt% of the untreated sample, FR-SH.

According to the results in Table 2, the Mg²⁺ content of FNRL was decreased from 657 to 294 ppm after the fresh latex was preserved with NH₄OH. This was due to the complex formation of magnesium ammonium phosphate (MgNH₄PO₄) when magnesium and phosphate ions of the fresh latex reacted with ammonia ion. The Mg²⁺ content of FR-NH₃ DPNR samples tended to reduce with increasing EDTA-4Na dosage, resulting in the lowest Mg²⁺ content (89 ppm) at 0.20 wt% EDTA-4Na. The Mg²⁺ content of FR-SH sample was slightly reduced compared to that of the starting material (FNRL). Again, the Mg²⁺ content of the nonammonia latex tended to decrease with increasing EDTA-4Na content; that is, the lowest Mg²⁺ content (289 ppm) was found in the latex treated with 0.20 wt% EDTA-4Na. The

results demonstrated that the magnesium ions present in the fresh NR latex could be scrapped by EDTA-4Na chelating agent under the same conditions used for deproteinization.

Effect of different EDTA salt complexes on deproteinization of nonammonia and ammonia NR latices was studied. As shown in Table 3, the highest deproteinization efficiency was observed with EDTA-4Na and tended to be lower with decreasing Na number in the complex. The lowest nitrogen contents of 0.008 wt% and 0.005 wt% were obtained for FR-NH₃ DPNR and FR-SH-DPNR treated with 0.10 wt% EDTA-4Na, respectively. EDTA-1Na, EDTA-2Na, and EDTA-3Na could also remove proteins by denaturation. The highest efficiency of EDTA-4Na on deproteinization could be due to its higher solubility in water compared to the other EDTA complexes. Removal of proteins in NR by EDTA suggested that most proteins present in fresh NR are attached to the rubber via weak attractive forces, which can be disturbed by chelation. Huang and coworkers reported that the coordination of metal ion with protein was destroyed by scrapping of metal by EDTA resulting in the denaturation of NR proteins [13].

Removal of divalent metal ions can result in denaturation of the proteins associated with the latex particles due to disturbance of the protein's structure [14]. The highest efficiency of EDTA-4Na on deproteinization of NR followed by EDTA-3Na, EDTA-2Na, and EDTA-1Na, respectively, can be explained by the chemical characteristics of the different EDTA complexes. EDTA in different forms have different capability on chelating divalent metal ions. According to

TABLE 3: Nitrogen content of FNR and deproteinized FNR prepared using various EDTA complexes at 0.10 wt%.

Samples	Preservatives	pH	0.1 wt% EDTA complexes	Nitrogen content (wt%)
FNR	—	6.5	—	0.638
FR-NH ₃	Ammonia	10	—	0.587
FR-SH	SH	10	—	0.551
FR-NH ₃	Ammonia	10	EDTA-1Na	0.029
FR-NH ₃	Ammonia	10	EDTA-2Na	0.022
FR-NH ₃	Ammonia	10	EDTA-3Na	0.013
FR-NH ₃	Ammonia	10	EDTA-4Na	0.008
FR-SH	SH	10	EDTA-1Na	0.022
FR-SH	SH	10	EDTA-2Na	0.012
FR-SH	SH	10	EDTA-3Na	0.009
FR-SH	SH	10	EDTA-4Na	0.005

FIGURE 2: Chemical structures of EDTA.

their chemical structures (see Figures 2 and 3), EDTA-4Na can chelate two divalent metal ions on stoichiometry basis. Lower chelating efficiency is expected for EDTA-3Na, EDTA-2Na, and EDTA-1Na, respectively. Solubility of the EDTA complexes in water also follows this order where EDTA-4Na showed the highest solubility. In addition, solubilization of EDTA-4Na led to pH between 10 and 11, which was very close to the pH of the NR samples while solubilization of EDTA-3Na, EDTA-2Na, and EDTA-1Na resulted in solution with less alkaline pH, respectively. Altogether, this resulted in the highest deproteinization efficiency of EDTA-4Na as observed in this study.

3.2. Determination of Proteins Pattern of DPNR-EDTA. Protein patterns in the cream fractions of NR samples deproteinized by different forms of EDTA were determined using SDS-PAGE.

Figures 4 and 5 show the protein patterns in the FNRL and deproteinized ammonia and nonammonia NR samples

separated according to their molecular weight. Overall treatment with EDTA under the experimental conditions led to decomposition of proteins to fragments with lower molecular weights. The protein patterns of the controls (FR-SH and FR-NH₃ FNRL) are shown in lane 1. The major bands at 14.4 and 25 kDa are known to be derived from proteins covering the surface of the rubber latex particle [15]. Slight degradation of proteins was observed in FNRL treated with EDTA (in lane 3). Distinct decomposition of proteins was found in DPNR with EDTA-4Na, EDTA-3Na, EDTA-2Na, and EDTA-1Na which resulted in protein bands with lower molecular weight and smear. No significant difference was observed between samples treated with different forms of EDTA complexes.

3.3. FT-IR Spectra of DPNR-EDTA-4Na. The residual proteins of ammonia and nonammonia fresh latices treated with 0.10 wt% EDTA-4Na were analyzed by FT-IR (Figure 6). For fresh NR latex, the clear peak at 3280 cm⁻¹ assigned to NH stretching was found indicating the existence of proteins or

FIGURE 3: Mechanism of chelation reaction with metal ions and metal ions on protein: (a) mechanism of chelation reaction; (b) metal ions in the protein layer was scraped by EDTA.

FIGURE 4: SDS-PAGE of redispersed cream fraction from deproteinized nonammonia FNRL with EDTA complexes. FN-SH samples were incubated with 0.1 wt% EDTA in different forms in the presence of 1 wt% SDS for 1 h. Lane 1: FR-SH, lane 2: mixture of FR-SH and EDTA-4Na without centrifugation, lane 3: washed FR-SH with SDS followed by centrifugation, lane 4: FR-SHDPNR-EDTA, lane 5: FR-SHDPNR-EDTA-1Na, lane 6: FR-SHDPNR-EDTA-2Na, lane 7: FR-SHDPNR-EDTA-3Na, and lane 8: FR-SHDPNR-EDTA-4Na.

peptides. After deproteinization with 0.10 wt% EDTA-4Na, both FR-SH DPNR-EDTA-4Na and FR-NH₃ DPNR-EDTA-4Na show their peak at 3320 cm⁻¹ instead of 3280 cm⁻¹. The newly appeared band around 3320 cm⁻¹ in the purified NR was assigned to the NH stretching of oligopeptides such as dipeptide [5, 16, 17]. The absence of NH stretching band in FR-SH DPNR-EDTA-4Na and FR-NH₃ DPNR-EDTA-4Na indicated that the amount of residual proteins in these samples was less than the detection limit of FT-IR analysis.

3.4. Physical Properties of DPNR. Physical properties of the DPNR prepared using the two different preservative systems with 0.10 wt% EDTA-4Na are compared with relevant international specifications according to the Standard Thai Rubber STR5L in Table 4.

The dirt content, volatile matter content, ash content, nitrogen content, and Mooney viscosity of both deproteinized NR samples were conformed to the specifications.

FIGURE 5: SDS-PAGE of redispersed cream fraction from deproteinized ammonia FNRL with EDTA complexes. FN-NH₃ was incubated with 0.10 wt% of EDTA in different forms for 1 hour at room temperature in the presence of 1 wt% SDS. Lane 1: FR-NH₃, lane 2: mixture of FR-NH₃ and EDTA-4Na without centrifugation, lane 3: washed FR-NH₃ with SDS followed by centrifugation, lane 4: FR-NH₃DPNR-EDTA, lane 5: FR-NH₃DPNR-EDTA-1Na, lane 6: FR-NH₃DPNR-EDTA-2Na, lane 7: FR-NH₃DPNR-EDTA-3Na, and lane 8: FR-NH₃DPNR-EDTA-4Na.

Exception was found for the PRI; that is, the PRI values for FR-SH DPNR-EDTA-4Na and FR-NH₃ DPNR-EDTA-4Na were 40.5 and 37.6, respectively. The results imply that the two DPNR samples possessed relatively low thermal oxidative aging resistance. This could be explained by the low protein content in the samples because it has been reported that natural proteins or amino acids in NR could act as natural antioxidant [18]. The presence of remnant oligopeptides or small protein fragments in DPNR and the nearly complete removal of proteins by surfactant washing could be assessed indirectly by measuring the resistance to thermal oxidative aging represented in terms of plasticity retention index (PRI).

4. Conclusions

The deproteinized natural rubber (PDNR) was successfully prepared in the latex stage for both preservative systems by

FIGURE 6: FT-IR spectra of DPNR-EDTA-4Na. (a) FNR, (b) FR-SH DPNR-EDTA-4Na, and (c) FR-NH₃ DPNR-EDTA-4Na.

TABLE 4: Physical properties of DPNR.

Properties	Samples		
	Standard of STR 5L	FR-SH DPNR-EDTA-4Na	FR-NH ₃ DPNR-EDTA-4Na
Dirt content (wt%)	0.04 max	0.004	0.003
Volatile matter content (wt%)	0.8 max	0.03	0.03
Ash content (wt%)	0.4 max	0.11	0.13
Nitrogen content (wt%)	0.6 max	0.005	0.008
Initial Wallace plasticity (P_0)	35 min	55.5	50.0
Plasticity retention index (PRI)	60 min	40.5	37.6
Mooney viscosity, ML(1 + 4) 100°C	—	67.0	66.3
Color (Lovibond)	6.0 max	1.0	1.5

using EDTA-4Na in the presence of surfactant. The optimum concentration of EDTA-4Na was 0.1 wt% while EDTA-1Na, EDTA-2Na, and EDTA-3Na could also remove protein but with lower efficiency. The magnesium ion content of the FNRL was reduced with increasing EDTA-4Na concentration. The nonammonia preservative has no strong smell, is free of nitrosamine, and is nontoxic to cell with adjustable pH

(8–11). These properties are considered useful for application in producing healthcare products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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