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The effect of remaining SDS on protein release in later leaching steps of deproteinized natural rubber

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Abstract

The effect of residual sodium dodecyl sulfate (SDS) used in the washing steps on further leaching of proteins from deproteinized rubber films was investigated. High-ammonia natural rubber latex (HANRL) was diluted with a 0.01% w/v potassium hydroxide solution to obtain 30% dry rubber content (DRC) solution referred to as diluted NRL. Untreated NRL was directly cast in a film (sample 0). Sample 1 was prepared from diluted NRL centrifuged at 5000 rpm for 1 min. Sample 1-2 was prepared by washing Sample 1 with a 0.5 %wt SDS solution. Sample 1-3 was prepared by washing Sample 1-2 with a 0.1%wt SDS solution. After that, they were cast into films. The dried films were checked for extractable protein (EP) using a modified Lowry method and nitrogen content determination according to SMR: Bulletin No.7-1992. The EP content of the Sample 1 film was less than the untreated (Sample 0), but the EP content was increased after washing with a 0.5 %wt SDS solution and then it decreased slightly after further washing with a 0.1 %wt SDS solution. In contrast, the nitrogen content and FTIR spectra confirmed that the total protein content of the films decreased when they underwent more treatment. This finding suggested that although overall protein content decreased, the remaining SDS on the films could facilitate further leaching of EP from the film surface when it was in contact with extraction medium (PBS or synthetic sweat) under conditions of shear. The use of this deproteinized film should, therefore, be done with care.

Keywords: Deproteinization, Extraction, Natural rubber latex, Protein, SDS

1. Introduction

Natural rubber (cis-1,4-polyisoprene) is a natural polymer from rubber trees. It is a colloidal system composed of hydrocarbon, protein and phospholipid. Natural rubber latex usually has dry rubber content (DRC) about 30-33% before it is centrifuged to yield concentrated latex and skim latex with DRC of 60% and 4-6%, respectively [1]. Concentrated latex is normally preserved with 0.7%wt ammonia, so it is called high ammonia natural rubber latex (HANRL). HANRL is a raw material for the production of many products such as gloves, condoms and balloons. However, the applicability of products from this latex is in some degree limited because some people are allergic to proteins in the rubber [2] with a prevalence of 1-6% in the general population and 8-16% in medical personnel [3]. Taking that into account, there are many methods in deproteinization, i.e. reduction of the extractable protein (EP) content in natural rubber, such as irradiation, enzymatic treatment, saponification, chlorination and denaturation. In irradiation method, protein chains in natural rubber could be cut by ^{60}Co gamma to small molecules so it can be removed more easily from rubber [4-5]. Papain [6] and alkali protease [7-8] were applied in enzymatic depolymerization of

proteins. However, enzymes are also proteins which might be allergic to people who are prone to protein allergy.

According to the new model of rubber particles, their surfaces are covered with a layer of both proteins and phospholipid, so saponification could be applied to remove phospholipid first to interrupt the attraction of proteins with the surface. KOH was used in saponification and it was found that protein content was reduced from 68 $\mu\text{g/g}$ to 10 $\mu\text{g/g}$ [9]. In contrast, chlorination could strengthen the attachment of proteins to natural rubber film. Soaking natural rubber film in hydrochloric acid solution (HCl) was reported to reduce EP from 1600 $\mu\text{g/g}$ to 200 $\mu\text{g/g}$ [10].

Previously, using sodium dodecyl sulfate (SDS), which is an anionic surfactant together with urea in denaturation, could reduce the protein content in natural rubber latex from 0.38 to 0.005 wt% [11-13]. Moreover, natural rubber could be free of protein when polar organic solvent together with SDS were applied [14]. During deproteinization, it is possible that the latex system may lose stability, so adding a surfactant is then necessary to sustain the colloidal stability. Because of its low cost and high availability in the market, SDS which is an anionic surfactant is usually chosen to stabilize the colloidal systems including rubber latex. However, we expect that applying a surfactant, which in this

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case is SDS, to the rubber latex may have a side effect which is facilitating the release of proteins in later steps of leaching. This has not been reported before and it is worth investigating in more detail. Thus, in this work the effect of remaining SDS in later leaching steps of the deproteinized natural rubber was studied.

2. Research methodology

2.1 Material

2.1.1 Material used in washing steps

High-Ammonia Natural Rubber Latex (HANRL) was obtained from Rubber Research Institute of Thailand. Sodium dodecyl sulfate (SDS; $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$) was obtained from Ajax Finechem Pty. Ltd. Potassium hydroxide (KOH) was obtained from MERCK, Germany.

2.1.2 Material used to check EP content

Reagent A was prepared by dissolving 2.22 g of sodium carbonate (Na_2CO_3 , Ajax Finechem Pty. Ltd.), 0.18 g of sodium tartrate ($\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$, QR&C Pty Ltd., New Zealand) and 0.44 g of sodium hydroxide (NaOH, MERCK, Germany) in water to make 100 ml.

Reagent B was prepared by dissolving 7 g of copper (II) sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Suksapan, Thailand) in water to make 100 ml.

Reagent C was prepared by mixing 1 ml of Reagent B and 150 ml of Reagent A.

Reagent D was prepared by diluting 1 part of Folin's reagent (Lobachiem, India) with 1 part of water.

Phosphotungstic acid (PTA) was purchased from HiMedia Laboratories Pvt Ltd., India. Sodium deoxycholate (DOC) was from Sigma Aldrich, USA, trichloroacetic acid (TCA) was from Fisher Scientific, UK and phosphate buffered saline (PBS) as extraction buffer was purchased from AMRESCO, USA. Lastly, sodium chloride (NaCl) and sodium hydrogen carbonate (NaHCO_3) were obtained from Ajax Finechem Pty. Ltd.

2.2 Protein extraction of HANRL by washing in batch process

In the experiment, the high-ammonia natural rubber latex (HANRL) was diluted with 0.01% w/v potassium hydroxide (KOH) solution to obtain latex with about 30% DRC. This latex is called "diluted NRL". It was casted directly into films (step 0). Other samples were prepared in many steps. Sample 1 was prepared by centrifugation at 5000 rpm for 1 min. Sample 1-2 was prepared by washing the cream fraction of Sample 1 with 0.5% wt SDS solution and sample 1-3 was prepared by washing Sample 1-2 with 0.1% wt SDS solution. After that they were casted into films as summarized in Figure 1.

2.3 Determination of the extractable protein (EP) content by modified Lowry method

The determination of the EP content was carried out according to ASTM D5712.

2.3.1 Extraction procedures

The extraction media used in this study were PBS buffer and synthetic sweat. To prepare 0.3% w/v PBS buffer, 3 g of PBS was dissolved in 1000 ml of distilled water. Synthetic

sweat was a mixture of $25.9 \times 10^{-3} \text{ M}$ of NaCl and $1.07 \times 10^{-3} \text{ M}$ of NaHCO_3 [15].

A rubber specimen with a known weight was immersed in 2 ml of different extraction medium and the mixture was shaken at 120 rpm for 120 min.

2.3.2 Acid precipitation

A rubber specimen was removed and only 1 ml of the remaining solution from section 2.3.1 was transferred to a polypropylene tube. It was centrifuged at 3600 rpm for 15 min. A total of 0.1 ml DOC was added, mixed thoroughly, and kept for 10 min. Next, a total of 0.2 ml of freshly prepared solution of 50:50 TCA and PTA was added. The mixtures were mixed well and allowed to stand for 30 min for proteins to be precipitated. The acid precipitate was obtained by centrifugation at 12400 rpm for 15 min. A total of 0.6 ml of 0.2 M NaOH solution was then added to each tube to redissolve the precipitated protein completely and obtain a clear solution.

2.3.3 Extractable protein determination

A total of 0.625 ml of reagent C was added to the extract specimen. The solution was mixed well and kept for 15 min at room temperature. A total of 0.075 ml of reagent D was added to each of them and it was immediately and thoroughly mixed and left for 30 min at room temperature. The absorbance of the final assay mixture was measured at 750 nm. The protein concentration of the extract in $\mu\text{g}/\text{ml}$ (C) can be calculated when UV absorbance of the sample was measured and compared with a calibration curve using ovalbumin as a protein standard. The amount of protein was reported as EP content (mg/dm^2), which is defined in Equation (1).

$$\text{Extractable protein (EP)} = \frac{C \times V \times F}{S} \quad (1)$$

Where, V is the volume of extraction buffer in ml, F is the dilution factor, and S is the surface area in dm^2 of the rubber specimen.

2.4 Characterization of deproteinized rubber by FTIR

Functional groups of deproteinized rubber were characterized by Fourier transform infrared spectroscopy (FTIR) in the mode of Attenuated Total Reflection (ATR). The results were obtained for every 2 cm^{-1} .

2.5 Nitrogen content

The nitrogen content in rubber was measured according to SMR: Bulletin No.7-1992.

3. Results and discussion

Protein extraction of untreated HANRL (diluted NRL) by batch process was done. The EP content obtained from Modified Lowry method when PBS buffer was used as extraction medium was shown in Figure 2.

As can be seen, the EP content of the sample casted directly to the film (sample 0) was about $22 \text{ mg}/\text{dm}^2$. The value dropped to $15 \text{ mg}/\text{dm}^2$ after centrifugation (sample 1) and then increased to $35 \text{ mg}/\text{dm}^2$ after washing with 0.5% SDS (sample 1-2) and dropped significantly after washing again with 0.1% SDS solution (sample 1-3).

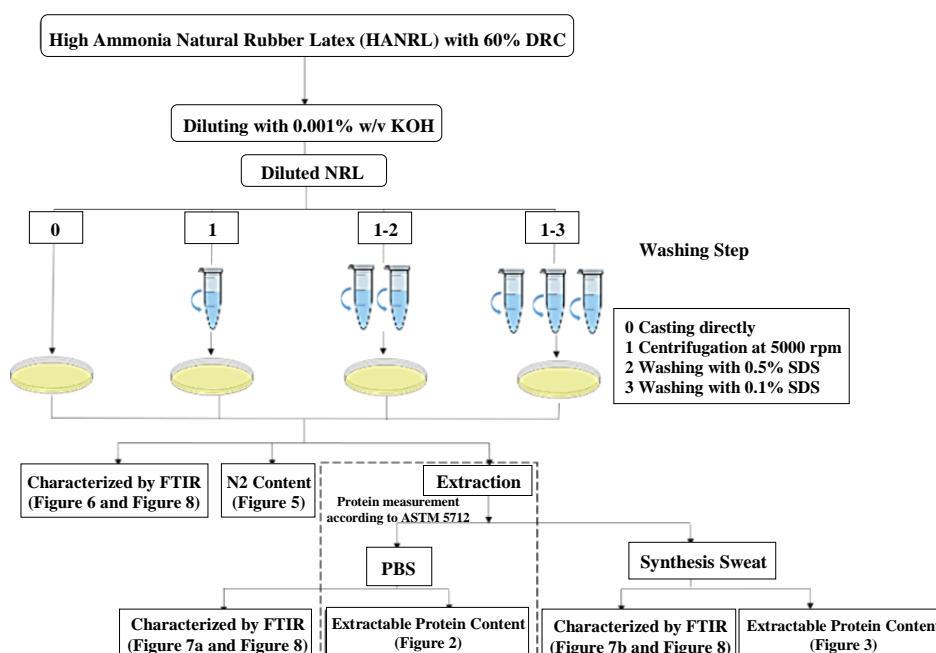


Figure 1 Schematic representation of experimental procedure

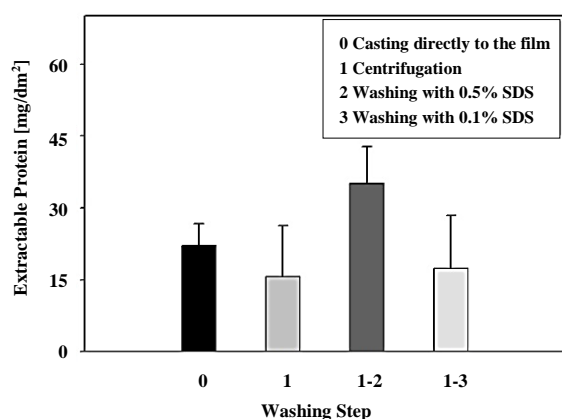


Figure 2 EP content of NRL when PBS buffer was used as extraction medium

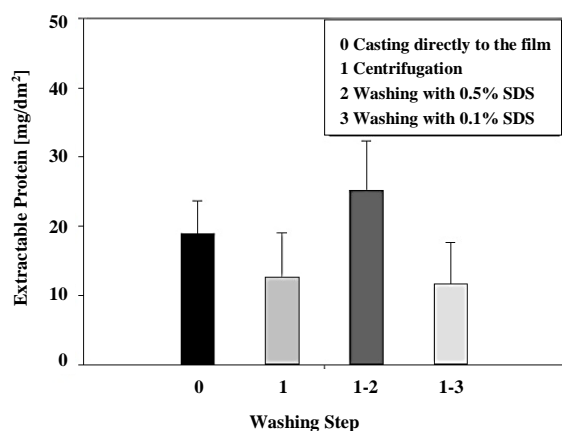


Figure 3 EP content of NRL when synthetic sweat was used as extraction medium

The EP content when synthetic sweat was used as extraction medium was comparatively shown in Figure 3. It

can be seen that the EP content of the film of untreated rubber (sample 0) is about 19 mg/dm² and it dropped to 12 mg/dm² after centrifugation (sample 1). After washing with 0.5% SDS (sample 1-2), EP content increased to 25 mg/dm² and dropped to 11 mg/dm² after washing again with 0.1% SDS solution (sample 1-3).

EP contents in both cases (Figures 2 and 3) show the same trend. The hypothesis of mechanism of protein extraction in each step was, therefore, proposed in Figure 4. It was possible that during centrifugation, some amount of proteins could be removed from the rubber particle surface because of the vigorous shear force exerted by the water in the centrifuge. When treated with SDS after the centrifugation (Steps 1-2), SDS molecules were expected to be attached to proteins on the rubber surfaces. Wei *et al.* studied the mixing of SDS at different concentration with protein BSA (Bovine Serum Albumin) solution. In some range of SDS concentrations, it was found that the surface tension of the mixture remained constant upon increasing SDS concentration. The finding suggested that SDS molecules were attached to BSA chains possibly by hydrophobic interactions among them rather than stayed at the air-water interface. The Fourier transform-Raman spectrum also suggested the unfolding of proteins, i.e. the change of protein conformation from α -helix to random coil [16]. The unfolding of proteins was also expected in our case where SDS could interact and be attached to the proteins on rubber surfaces. This could decrease the attractive forces between hydrophobic parts of proteins and rubber so proteins could be removed more easily from the rubber particles.

When the film of SDS-treated latex was leached with PBS buffer, higher amount of proteins was also leached. In addition, if the washing step was extended to include Step 3, treating with lower concentration of SDS solution, EP content of Sample 1-3 was still greater than only centrifugation (Sample 1) but slightly less than Sample 1-2 because the amount of proteins on the surface of the film was now less. The finding implied the effect of remaining SDS on leaching more proteins from the rubber in later steps of washing. The results for the synthetic sweat also showed the same finding. This raises a concern that proteins may be

leached more from the products if the products are in contact with sweat in the situation of a great shear force.

It is interesting to check whether the amount of proteins was truly reduced when applying more washing steps. To do so, the nitrogen content was tested. Figure 5 shows the results of nitrogen content determined according to SMR: Bulletin No.7-1992 in NRL film. Nitrogen content reflects the quantity of amino groups which corresponds to the amount of proteins in the system. It was found that nitrogen content was decreased from 0.30%wt to 0.28%wt after centrifugation and slightly decreased when further washing with SDS solution.

However, nitrogen contents in the sample 1-2 and sample 1-3 were approximately equal to 0.22%wt. The decrease of nitrogen content was not seen possibly because Sample 1-3 was prepared newly, not derived from Sample 1-2. They were not actually the same sample undergoing successive steps.

In addition, FTIR spectra were investigated to confirm our results. The absorption bands around 3,280 and 1,440 cm^{-1} , which correspond to -NH stretching and -NH bending, respectively, represent mono- or dipeptides in amino group of protein. Therefore, FTIR spectra in Figure 6 confirmed that the amount of protein in films really decreased when they underwent more treating steps. Additionally, the peak at 1229 cm^{-1} corresponding to S=O stretching vibration of sulphate group from SDS was observed which implied the attachment of SDS molecules to the rubber sample.

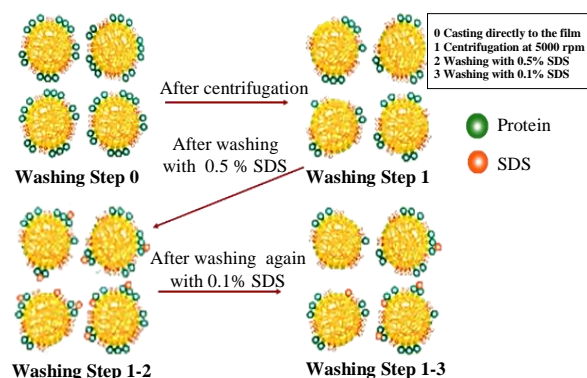


Figure 4 Hypothesis of mechanism of protein extraction in each step

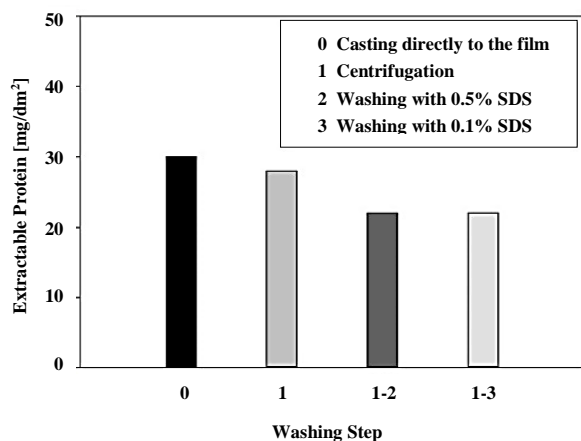


Figure 5 The nitrogen content of rubber films after each step of washing

Moreover, the samples after being leached by PBS buffer and the synthetic sweat were also investigated for their functional groups. Figure 7 shows FTIR spectra of these rubber films. The similar FTIR patterns were observed for both samples. The peak at 3,400 cm^{-1} representing hydroxyl group in sample 1-2 and sample 1-3 was observed. Even though this hydroxyl group was not part of SDS molecules, the higher peak for samples treated with SDS might imply the hydrophilicity of the polar part of SDS molecules. SDS molecules possessed both polar and non-polar parts. While the non-polar part interacted with rubber particles, the polar one could interact with water molecules, which might be present in the form of moisture on the surface of the rubber

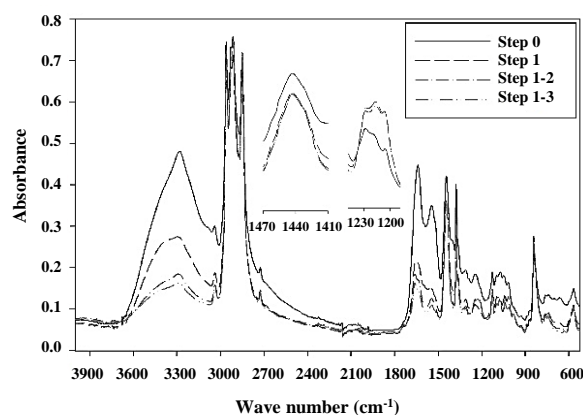


Figure 6 Fourier transform infrared spectra of NRL after washing in various steps.

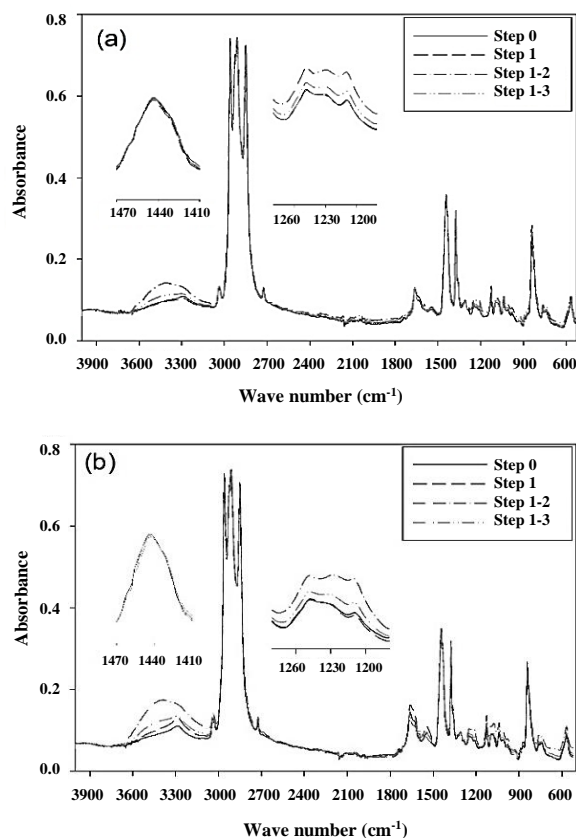


Figure 7 Fourier transform infrared spectra of NRL after extraction with (a) PBS and (b) synthetic sweat

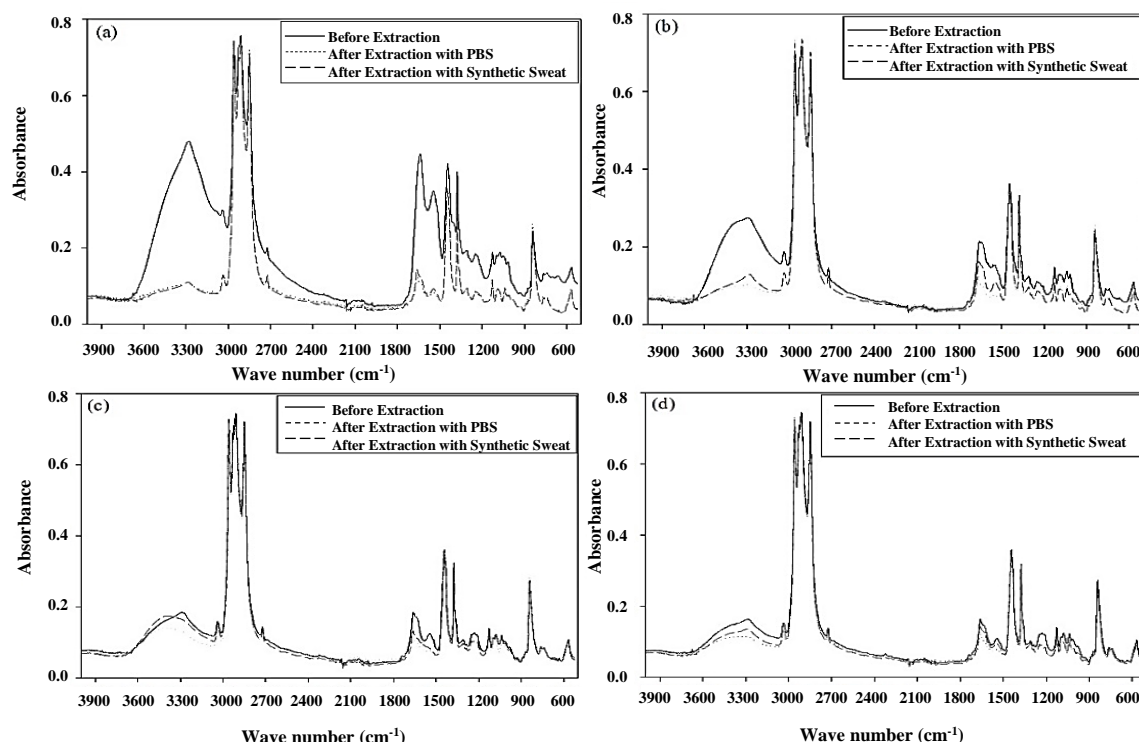


Figure 8 Fourier transform infrared spectra of NRL before extraction and after extraction with PBS and synthetic sweat were compared for the samples obtained from different treatment. (a) No treatment, (b) Step 1 only, (c) Steps 1-2 and (d) Steps 1-3

films. The topic regarding the degree of hydrophilicity of rubber film surface should be investigated further in the future.

Plotted differently in Figure 8, FTIR spectra of samples extracted by PBS buffer and the synthetic sweat could be compared more clearly. It was observed that even though the spectra looked similar for both PBS buffer and the synthetic sweat, small discrepancies could be seen. It seemed that smaller peaks were seen for PBS-extracted samples at 3280 cm^{-1} , which represented functional groups of proteins. This indirectly implied that PBS was more effective in protein leaching than synthetic sweat. It was reported that treating with urea and washing with SDS could free proteins from natural rubber particles effectively [12]. However, in this work, using SDS in washing step caused more release of protein.

This was not the first time that the effect of remaining surfactant was observed. In our previous work, the deproteinization of skim natural rubber latex was performed by using PEG as surfactant in both batch and continuous flow processes [17]. The results from the batch extraction showed that PEG molecules were attached to the rubber sample. The film made of latex treated with higher concentration of PEG yielded higher amount of proteins released when extracted with various extraction medium. Therefore, the use of surfactant-treated deproteinized film should be done with care.

4. Conclusion

This work discussed the effect of remaining SDS on protein release in later leaching steps of deproteinized rubber films. It was obviously seen that more steps of treatment could reduce the total amount of proteins in the rubber films as suggested by the nitrogen content results as well as by the

FTIR spectra of the rubber films before and after each treatment. However, the decrease of total protein content might not well indicate lower probability for a person to be allergic to proteins when in contact with surfactant-treated deproteinized rubber product. This research suggested that sodium dodecyl sulfate (SDS), once attached to the rubber surfaces in the step of washing latex, could facilitate the denaturation of proteins when the rubber film was leached with an extracting medium resulting in higher extractable proteins (EP) content. The tests were performed for both PBS buffer and the synthetic sweat and the results from both tests were quite the same. It should be noted that the condition for the extraction was that the great shear force was applied when mixing. Therefore, the worst case scenario was presented here in this research.

When the surfactant-treated deproteinized products are used, if they are subjected to a great shear force together with the extracting medium such as human sweat, more amount of proteins may be leached out of the products than expected. The use of such products, thus, should be done with care. The future work on how to reduce the amount of surfactants attached to rubber particles after the treatment should be investigated in order to know the threshold above which the side effect would be observed. In another aspect, the methods that could free proteins completely are of great interest so that the presence of SDS or any other surfactants would not yield such negative effect.

5. Acknowledgments

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