Studies on the effect of purified natural rubber latex and accelerators on rubber allergens in natural rubber dipping product

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Abstract. Natural rubber (NR) glove is a worldwide use product of NR latex, which is mainly used for protective purposes. However, allergic reactions to NR gloves causing by the residual proteins in NR latex and chemical used in the manufacturing process, which are called as rubber allergens, are still a significant concern. Thus, the present work is an attempt to minimize such the rubber allergens from NR dipping products, herewith, finger cot is used as a model for this study. Purified NR latices were prepared by an urea treatment and saponification method, called as deproteinized NR (DPNR) and saponified NR (SPNR), respectively. Both of DPNR and SPNR were found to have low nitrogen content. Fourier-transform infrared spectroscopy (FTIR) was used to identify functional groups as a result of the absence of the amine functional group, which can be referred to proteins. For a compounded NR, the various purified NR latices and rubber accelerators were used in the compounded formula. The existing and residual proteins of each sample were analysed by Bradford’s assay and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method, respectively. The DPNR and SPNR were found to contain lower protein profiles. The residual rubber accelerators released into artificial sweat were extracted and analysed by high-performance liquid chromatography (HPLC) technique.

1. Introduction
Natural rubber (NR) from Hevea brasiliensis tree is an important naturally occurring rubber source in industry due to its outstanding properties, i.e. excellent green strength, excellent tear resistance and low heat build-up. There are several dipping products made from NR latex such as gloves, condoms, pacifier and catheter, etc. However, the usage of these products sensitize the users to NR allergy, which reaction can vary from mild to severe symptoms. The most common and least-threatening reaction type is a non-immunological irritant contact dermatitis. For more-severe type, cell-mediated contact dermatitis or type IV allergy is an allergic reaction to chemical used in manufacturing of NR products. Rubber accelerator, especially thiurams and dithiocarbamates groups, is one of a chemical used that have been reported as a rubber allergen [1-2]. Another severe allergic reaction type that can develop to a life-threatening symptom cause by latex hypersensitivity is called immunoglobulin E (IgE)-mediated latex allergy or
type I allergy. Due to the ability of some proteins, which is one of a non-rubber composition, in NR latex can trigger type I immune responses individually sensitive people. There are many allergic proteins in NR latex and the most common ones were rubber elongation factor (REF) (Hev b 1), beta-1,3-glucanase (Hev b 2), Small rubber particle protein (SRPP) (Hev b 3), Acid latex protein (Hev b 5), Hevein (Hev b 6.02) and class I chitinase (Hev b 11) [3-4].

Therefore, this research aims to study the two important rubber allergens that can cause NR allergy, which are residual proteins and accelerators used in a sulphur vulcanization system of NR dipping product. Purified NR latex was prepared and subjected to a study of the effect of purified method on the minimization of allergic proteins in NR, while NR dipping product vulcanized with dithiocarbamate accelerators, zinc diethyldithiocarbamate (ZDEC) and zinc dibutylidithiocarbamate (ZDBC), were prepared to investigate a releasing of residual accelerators to artificial sweat.

2. Experimental
NR latices in this experiment were prepared from concentrated NR (CNR) latex with 61% dry solid content (DRC). Deproteinized NR (DPNR) were prepared from deproteinization process by urea and the presence of sodium dodecyl sulphate (SDS) as a surfactant under ambient condition [5]. Saponified NR (SPNR) were prepared from saponification process using NaOH and the presence of Triton-X® 100 as a surfactant under 70°C [6]. For the compounded NR, the ingredients are presented in the Table 1.

| Formula | CNR | KOH | K-laurate | Sulphur | ZDEC | ZDBC | Winstay L | ZnO |
|---------|-----|-----|-----------|---------|------|------|-----------|-----|
| VZDEC   | 100 | 0.3 | 0.2       | 0.5     | 0.75 | -    | 0.5       | 0.25|
| VZDBC   | 100 | 0.3 | 0.2       | 0.5     | -    | 0.75 | 0.5       | 0.25|

FTIR (JASCO FT/IR-4100) in transmission mode with film method, which samples were cast on KBr plate of liquid cell was used to determine the functional groups, which can be referred to a protein, in purified samples. In the same way, nitrogen content of purified samples was determined using the combustion method via nitrogen analyser (LECO FP-528). To study the existing rubber allergens, the proteins in both purified and vulcanized latices were extracted and observe protein pattern by a SDS-PAGE method [7]. After the latices was obtained, proteins were extracted by using acetone/methanol as precipitated co-solvent, while the extractable proteins from the dried NR samples were stirred into SDS aqueous solution for 12 h then the proteins were precipitated by co-solvent system. The residual dithiocarbamate accelerators were extracted in an artificial sweat for 24 h under 37°C then converted into copper complexes before analysis by HPLC technique [8]. Chromatographic analyses were performed using HPLC (Dionex, Ultimate 3000) on a reverse phase column eluted with a solution of methanol and water in the ratio 90:10 (v/v) as a mobile phase.

3. Results and discussion
FTIR spectra of CNR and purified NR, DPNR and SPNR, were shown in Figure 1. The broad band centred at 3200 cm⁻¹ corresponding to –N-H stretching, which is the functional group of proteins, can be clearly observed in the CNR sample. However, the absence of this broad band was observed in DPNR and SPNR sample. It was also clear that nitrogen content of CNR, DPNR and SPNR samples were 0.399, 0.063 and 0.032% respectively. Indicating that the obtained DPNR and SPNR were purified latices that have lesser protein containing. Moreover, SPNR prepared from saponification method, which is a chemical reaction of base catalysed hydrolysis of ester and amide groups, was expected to show lower protein than urea-treatment, which is a physical interaction of urea-induced denaturation of protein, of DPNR.
The International Conference on Materials Research and Innovation (ICMARI)

IOP Conf. Series: Materials Science and Engineering 773 (2020) 012041 doi:10.1088/1757-899X/773/1/012041

Figure 1. FTIR spectra of rubbers from CNR, DPNR and SPNR lattices.

The extractable allergic protein, which is one of rubber allergens, from NR lattices and dried NR samples are shown in Figure 2. It was found that protein from CNR sample showed lower protein band than fresh NR (FNR) sample that showing the beta-1,3-glucanase, class 1 chitinase, SRPP and REF proteins. In SPNR samples, there are no detectable proteins. It can be indicated that SPNR prepared from saponification method is the highly purified NR that free from allergic proteins. Moreover, the latex and dried sample of the obtained vulcanized NR from CNR showed the same protein band around 14 kDa, which is REF protein.

Figure 2. SDS-PAGE profiles of extracted proteins from various NR samples, Lane b-g are originated from latex sample, while lane h-m are from dried rubber. Lane a) protein marker, b) fresh NR latex, c) CNR latex, d) VZDBC latex, e) VZDEC latex, f) DPNR latex, g) SPNR latex, h) dried fresh NR, i) dried CNR, j) dried VZDBC, k) dried VZDEC, l) dried DPNR and m) dried SPNR.
Figure 3. HPLC chromatograms of copper complex of ZDEC (left) and ZDBC (right) at λ 435 nm with the comparison of a) standard accelerators and b) accelerator-released into sweat of samples.

The residual accelerator-release into the artificial sweat was investigated by HPLC analysis. From Figure 3, copper complex ZDEC and ZDBC were observed at 1.3 and 5.7 mins respectively. The extent of ZDEC-release into artificial sweat, at pH 6.5 for 24 h under 37°C, from prepared dipping sample was 4.63 μg/g. Meanwhile, the releasing of ZDBC could not be found from the ZDBC-dipped sample. This implies that there is more occasion of the accelerator-release from a NR dipped product using ZDEC as an accelerator than ZDBC. Therefore, NR dipped products using ZDBC as an accelerator were recommended to use due to the non-released allergic accelerator that can expose to human skin.

4. Conclusions
In this research, DPNR and SPNR showed lower protein functional groups as a result of FTIR, and nitrogen content comparing to that of the CNR. SDS-PAGE analysis showed the lower allergic protein bands of CNR, DPNR and SPNR than FNR. Moreover, it can be concluded that the obtained SPNR is the highly purified NR that free from allergic proteins. Further studied on the released-accelerator of NR dipped product from HPLC technique, ZDBC could not be found from the artificial sweat extraction. Therefore, the prepared NR dipped product from purified latex and ZDBC accelerator, instead of ZDEC, was suggested to be a product of choice that can minimize the rubber allergens.

5. References
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Acknowledgement
The authors would like to acknowledge the financial support from the Centre of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Science, Research and Innovation, Mahidol University. The SDS-PAGE method were support with the helping of Mrs. Nuanwan Phunthanom at Institute of Molecular Biosciences, Mahidol University. Sincere appreciation is extended to the Thai Rubber Latex Group Public Company Limited for their kind support of NR latex.