The Potential of Enzyme in Reducing the Maturation Time and the Use of Ammonia in Concentrated Natural Rubber Latex

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Abstract. One of the most important parameters for indicating colloidal stability of concentrated natural rubber latex (CNRL) is the mechanical stability time (MST). The MST of CNRL after centrifugation is always low and slowly increases with storage time due to gradual hydrolysis of phospholipid on the surface of rubber particles into fatty acid soap. In this work, enzymatic approach was investigated to increase the colloidal stability of CNRL by using different grades and amounts of enzymes. The enzymes were tested with 2 grades of CNRL, which are the high-ammonia latex (HA latex) and low-ammonia latex (LA latex). We found that the MST of LA latex treated with 0.004% and 0.04% of Lipex reach the acceptable value (at least 600 sec.) within 14 and 10 days, respectively. In case of HA latex, the MST reached the acceptable value faster than that of LA latex (within 10 and 3 days at amount 0.004% and 0.04% of Lipex, respectively) while, the MST of commercial laurate-treated latex only increased slightly and could not pass the acceptable value after 31 days. In addition, the use of enzyme instead of laurate soap could reduce the use of ammonia in CNRL production.

1. Introduction

Fresh natural rubber latex (FNRL) tapped from rubber trees contain about 35% dry rubber content, which is unsuitable for producing rubber products. To increase the rubber content in latex, FNRL is usually converted into concentrated natural rubber latex (CNRL), which contains about 60% dry rubber content. The most popular commercial technique for producing CNRL is by centrifugation. There are two common grades of commercial latex, one is the commercial high-ammonia latex (commercial HA latex) and the other is the commercial low-ammonia latex with the presence of TMTD and zinc oxide (commercial LATZ latex). To preserve and stabilize the commercial HA latex, 0.65 wt% ammonia and 0.02-0.04 wt% ammonium laurate soap are added, while 0.25 wt% ammonia, 0.05 wt% ammonium laurate soap, 0.13 wt% TMTD and 0.13 wt% ZnO are added in commercial LATZ latex.

To ensure the latex stability of CNRL, the MST of above 600 seconds is generally specified in the standard Thai latex for sellable CNRL. However, the initial stability of CNRL freshly produced from the centrifugation process is low because the rubber particles are closer leading to an increasing...
attractive force and lower latex stability. Naturally, the MST of CNRL slowly increases with storage time due to gradual hydrolysis of phospholipid on rubber particles into fatty acid soap [1, 2, 3, 4]. Therefore, maturation of CNRL is necessary to allow the increase of MST. The practical method to expedite the increase of MST for commercial CNRL is by direct addition of negative ions such as laurate soap [5, 6] or other surfactants [7, 8] into CNRL. However, the increase of MST from this method leads to over-stabilization of the latex at long time, which would cause coagulation difficulty in the product forming processes [9]. To accelerate the MST of CNRL without over-stabilizing problem, a preliminary work in our group found that the enzyme lipase was more effective in increasing the MST of HA latex [10]. Moreover, the MST of HA latex reached a stable value after 10 days of storage time without causing an over-stabilized problem at long time. Therefore, lipase addition is a potential method to reduce maturation time in latex production. In this work, we expand the research scope by exploring the potential of different grades and contents of lipase in 2 different grades of CNRL including the high-ammonia and the low-ammonia grades, and compared the results to commercial HA latex and LATZ latex. The potential of lipase usage in reducing the use of ammonia and other preservatives is also discussed.

2. Experimental

2.1 Materials

In this study, the high-ammonia latex without stabilizer was denoted as HA latex and low-ammonia grade without stabilizer and secondary preservatives (TMTD/ZnO) was denoted as LA latex. The freshly produced HA latex and LA latex were purchased from Thai Eastern Rubber Co., Ltd. The commercial HA latex and LATZ latex were also provided from the same company. The lipase enzymes obtained from Novozyme Co., Ltd. and Reach Biotechnology Co., Ltd were denoted as Lipex and CNLP, respectively.

2.2 Preparation of CNRL treated with lipase

To study the effect of lipase, the CNRL samples were treated with 2 grades of lipase (Lipex and CNLP) at the concentration of 0.004% and 0.04% by volume of CNRL. The treated samples were kept at room temperature and measured for the MST values, total solid content (TSC), dry rubber content (DRC), alkalinity (%NH₃) and pH. The untreated CNRL and the commercial CNRL treated with ammonium laurate soap were used as reference.

2.3 Determination of Properties

The MST was determined by using Mechanical Stability Test Machine (Klaxon MST test apparatus) operating at 14,000 rpm following the ISO 35:2004 standard. The TSC was determined from the weight of dried latex at 70 °C for 16 hr according to the ISO124:2014 and the DRC was determined by acid coagulation before drying following the ISO 126:2005. The alkalinity as ammonia (%NH₃) was determined by titration with 0.1 M HCl according to ISO 125:2011.

3. Results and discussion

3.1 Effect of lipase grades on the MST of CNRL

In this part, 2 grades of CNRL were used in this study including the high ammonia latex (HA latex) and the low ammonia latex without TMTD and zinc oxide preservatives (LA latex). The MST of CNRL samples treated with 2 grades of lipase were compared with the untreated latex samples. The MST values were measured after 41 days of maturation and the results are shown in Fig. 1. As can be seen, the MST of CNRL samples treated with Lipex was higher than that treated with CNLP in both types of CNRL. This indicated that Lipex was more effective than CNLP in accelerating the hydrolysis of phospholipids in CNRL. Therefore, Lipex was further used for accelerating the MST of CNRL in this research. Other properties of the CNRL samples in Fig 1 were reported in Table 1. It was found that, the values of TSC, DRC, %NH₃ and pH were not significantly affected by lipase treatment.
This implied that lipase addition did not cause any adverse effect on the CNRL.

**Fig. 1** The MST (at 41 days) of untreated latex, CNRL treated with 0.004%CNLP and 0.004%Lipex

**Table 1** Other properties of CNRL samples at 40 days

| Sample                              | Property | TSC (%) | DRC (%) | %NH₃ | pH   |
|-------------------------------------|----------|---------|---------|------|------|
| Untreated LA latex                  |          | 61.02   | 60.40   | 0.23 | 9.28 |
| LA latex treated with 0.004%CNLP    |          | 61.68   | 60.42   | 0.25 | 9.33 |
| LA latex treated with 0.004%Lipex   |          | 61.93   | 60.37   | 0.25 | 9.26 |
| Untreated HA latex                  |          | 61.64   | 59.75   | 0.55 | 9.73 |
| HA latex treated with 0.004%CNLP    |          | 61.86   | 60.04   | 0.54 | 9.77 |
| HA latex treated with 0.004%Lipex   |          | 61.07   | 59.97   | 0.54 | 9.67 |

### 3.2 Effect of lipase concentration on MST of CNRL

In this part, Lipex was added to CNRL (LA and HA latex) in the amount of 0.004% and 0.04% by volume of CNRL and the MST values of CNRL samples were followed for 31 days. The MST values of the Lipex-treated samples were compared with the commercial latex samples maturated for the same time as shown in Table 2. It was found that the MST of Lipex-treated CNRL increased more rapidly than the commercial samples in both LA latex and HA latex. The MST of LA latex treated with 0.004% and 0.04% of Lipex reach the acceptable value (at least 600 sec.) within 14 and 10 days, respectively. In case of HA latex, the result showed the same trend but the MST reached the acceptable value faster (within 10 and 3 days at amount 0.004% and 0.04% of Lipex, respectively). On the other hand, the MST of commercial laurate-treated latex only increased slightly and could not pass the acceptable value after 31 days.
**Table 2** The MST (for 31 days) of commercial latex compared with latex treated with 0.004% and 0.04% Lipex

| Maturation time (days) | MST (sec.) | LA latex | HA latex |
|------------------------|------------|----------|----------|
|                        |            | commercial LATZ latex | Treated with 0.004% Lipex | Treated with 0.04% Lipex | commercial HA latex | Treated with 0.004% Lipex | Treated with 0.04% Lipex |
| 1                      |            | -        | 214      | 315      | -        | 270      | 396 |
| 3                      |            | -        | 364      | 426      | -        | 498      | 663 |
| 6                      |            | 85       | 447      | 545      | 86       | 555      | 774 |
| 10                     |            | 91.5     | 544      | 628      | 134.5    | 694      | 949 |
| 14                     |            | -        | **627**  | 591      | -        | 1002     | 1023 |
| 17                     |            | 119      | 620      | 841      | 164      | 975      | 1036 |
| 31                     |            | 137      | 675      | 851      | 195      | 983      | 1179 |

From these results, it indicated that, lipase was more effective than the commercial laurate soap in improving the latex stability. Besides, the higher concentration of Lipex (0.04%) was more effective than at 0.004% as the higher lipase content could accelerate faster hydrolysis of phospholipids.

### 3.3 Effect of ammonia content on MST of CNRL

The effects of ammonia content on MST of Lipex-treated latex were compared with that of commercial latex samples in Table 2. As can be seen, HA latex (higher ammonia content) possessed higher MST than the LA latex in all cases, indicating that the ammonia content strongly affected the increase in MST of the Lipex-treated samples. This indicated that the hydrolysis of lipids was faster under more alkali condition. Nevertheless, all Lipex-treated latex possessed much shorter maturation time than the commercial laurate-treated ones.

### 4. Summary

In this research, lipase treatment was an effective method in accelerating the MST of both low- and high-ammonia CNRL without affecting other properties of CNRL. The results showed that, the MSTs of lipase-treated CNRL samples were significantly higher than that of the commercial laurate-treated HA and LATZ latex indicating that lipase could effectively hydrolyze lipids and phospholipids on rubber particles. We found that the MST of LA latex treated with 0.004% and 0.04% of Lipex reach the acceptable value (at least 600 sec.) within 14 and 10 days, respectively. In case of HA latex, the MST reached the acceptable value faster than that of LA latex (within 10 and 3 days at amount 0.004% and 0.04% of Lipex, respectively). This indicated that the hydrolysis of lipids was faster under more alkali condition.

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6. References

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