Removal of Proteins from Natural Rubber with Urea and Its Application to Continuous Processes

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Received 25 June 2007; accepted 13 August 2007
DOI 10.1002/app.27236
Published online 6 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The removal of proteins from natural rubber through a batch process was studied by the incubation of the rubber latex with urea in the presence of sodium dodecyl sulfate. Under suitable conditions, the total nitrogen content of deproteinized natural rubber (DPNR) decreased from 0.38 to 0.02 wt % after incubation for 10 min; this was similar to that of the rubber deproteinized with a proteolytic enzyme for 12 h. For applications, continuous incubation and centrifugation were individually investigated by the use of a semicircular channel and a continuous centrifuge, respectively, to scale up DPNR preparation. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2329–2332, 2008

Key words: proteins; rubber; biopolymer

INTRODUCTION

The removal of proteins from natural rubber (NR) with urea through a continuous process is conducted to scale up deproteinized natural rubber (DPNR) preparation rapidly and efficiently. The proteins present on the surface of NR particles in the latex state may sometimes bring about a latex allergy mediated by type I immunoglobulin E[1–3] in sensitive individuals and a side reaction during the chemical modification of NR.[4] The proteins are either chemically bonded or physically held by the rubber particles.[5–7] The former is cleaved with a proteolytic enzyme such as alkaline protease,[5,8] and the latter is denatured with urea,[6,7,9,10] which may change the conformation of the proteins. For the continuous process, the proteins must be removed from the NR latex rapidly and efficiently. In previous work,[11–17] the removal of proteins was mainly performed in the latex state with a proteolytic enzyme that might decompose the proteins. In fact, it has already been reported that the total nitrogen content of NR can be reduced to less than 0.02 wt % after incubation with a proteolytic enzyme, which is about 1/20 of that of untreated NR.[5] However, the enzymatic deproteinization must be carried out in a batch system due to the long incubation time and strict temperature control. Therefore, it is quite important to develop a novel technique to appply to continuous process.

On the other hand, urea is known to change the conformation of proteins because of interactions between urea and proteins.[7] If the proteins are attached to the rubber particles via physical interactions but not chemical interactions, it may be possible to remove the proteins from NR in the latex state after denaturation of the proteins with urea rapidly and efficiently.

In this study, the removal of proteins from NR latex with urea as a denaturant in the presence of a surfactant was investigated through a batch process. Furthermore, this method through a batch process was applied to a continuous process to scale up DPNR preparation.

EXPERIMENTAL

The NR latexes used in this study were a fresh NR latex and a commercial high-ammonia natural rubber (HANR) latex. In the batch process, the incubation of the latex was performed with 0.1 wt % urea in the presence of 1 wt % sodium dodecyl sulfate (SDS; Kishida Co., Ltd., Osaka, Japan) at 303 K. The cream fraction was redispersed in a 1 wt % SDS solution to make a 30 wt % dry rubber content latex and washed twice by centrifugation to prepare urea-treated HANR (U-DPNR) latex and urea-treated freah NR (fresh U-DPNR) latex. The NR latex was also deproteinized by incubation of the latex with 0.04 wt % proteolytic enzyme (KP-3939, Kao, Tokyo,

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Journal of Applied Polymer Science, Vol. 107, 2329–2332 (2008)
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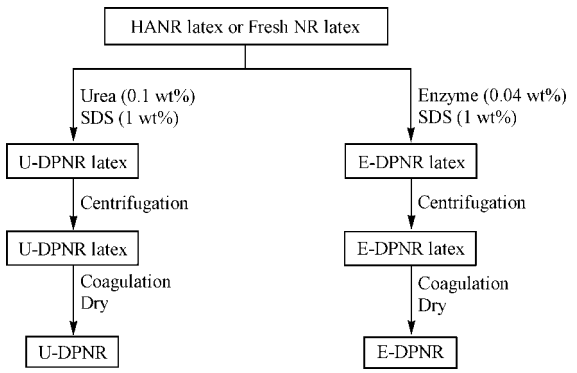

Figure 1 Schematic representation of the experimental procedure.

Japan) in the presence of 1 wt % SDS for 12 h at 305 K followed by centrifugation. The cream fraction was redispersed in a 1 wt % SDS solution to make a 30 wt % dry rubber content latex and was washed twice by centrifugation to prepare an enzymatically deproteinized HANR (E-DPNR) latex and enzymatically deproteinized fresh NR (fresh E-DPNR) latex. The rubber was recovered by centrifugation followed by coagulation with methanol and dried to a constant weight under reduced pressure at the ambient temperature. A schematic representation of the experimental procedure is shown in Figure 1.

As a model plant for the continuous incubation, one end of a semicircular channel, which was 50.4 m in length and 150 mm in diameter, was lifted to a height of 4.8 m to allow the latex to spontaneously flow. HANR latex containing 1.0 wt % SDS and 0.1 wt % urea flowed from the lifted end of the semicircular channel and was recovered at the lower end of the channel. The recovered latex was centrifuged three times to produce U-DPNR latex, which was coagulated with methanol and dried to a constant weight under reduced pressure at the ambient temperature.

Continuous centrifugation was investigated with an LRH410 purchased from Alfa Laval (Tokyo, Japan), after the batch incubation of HANR latex with 0.1 wt % urea in the presence of 1.0 wt % SDS.

TABLE I
Total Nitrogen Content and Incubation Time for HANR, Fresh NR, and DPNR

Specimen	Incubation time (min)	Total nitrogen content (wt %)
HANR	—	0.380
E-DPNR	720	0.017
U-DPNR	60	0.020
S-DPNR	60	0.028
Fresh NR	—	0.450
Fresh E-DPNR	720	0.014
Fresh U-DPNR	60	0.004
Fresh S-DPNR	60	0.088

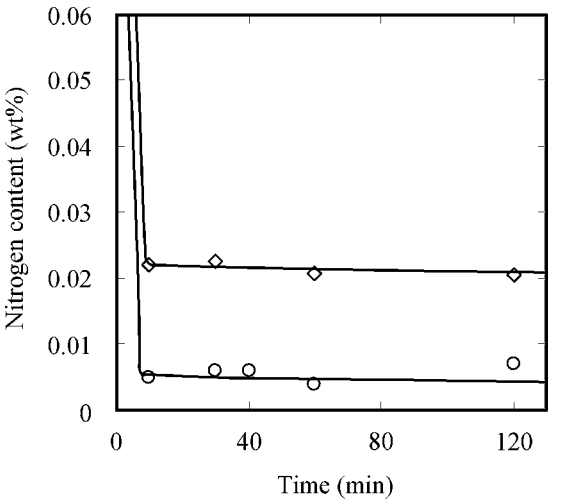

Figure 2 Total nitrogen content of (◇) U-DPNR and (○) fresh U-DPNR at 303 K versus the deproteinization time.

The measurement of the total nitrogen content of the rubbers was achieved by the Kjeldahl method, as described in the Rubber Research Institute of Malaysia (RRIM) test.[18]

RESULTS AND DISCUSSION

The total nitrogen content of HANR and DPNR, which is proportional to the content of proteins present in the rubber, is shown in Table I. The total nitrogen content of HANR decreased from 0.380 to 0.017 wt % after incubation with a proteolytic enzyme for 12 h under strict temperature control (E-DPNR), as reported in the previous study.[19] On the other hand, it was reduced to 0.020 wt % after incubation with urea at room temperature for 1 h (U-DPNR), being similar to that of E-DPNR. It is noteworthy that the incubation of fresh NR with urea for 1 h brought about a drastic reduction of the total nitrogen content to 0.004 wt %, which was one-third of the total nitrogen content of fresh E-DPNR. This may demonstrate that most proteins present in fresh NR are attached to the rubber with weak attractive forces, which can be detached with urea. The slightly higher total nitrogen content of U-DPNR prepared from HANR may indicate the strong attachment of the proteins to the gel fraction of NR increasing

TABLE II
Total Nitrogen Content and the Concentration of Urea for Fresh U-DPNR

Specimen	Concentration of urea (wt %)	Total nitrogen content (wt %)
Fresh U-DPNR	0.04	0.006
Fresh U-DPNR	0.1	0.005
Fresh U-DPNR	0.4	0.007
Fresh U-DPNR	1.0	0.012
Fresh U-DPNR	3.0	0.020

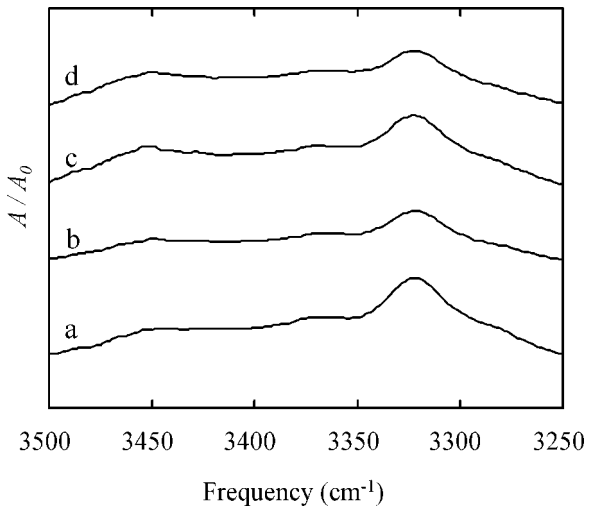

Figure 3 Fourier transform infrared spectra of fresh U-DPNR treated with various amounts of urea: (a) 0.05, (b) 0.1, (c) 0.5, and (d) 1.0 wt %.

during the preservation of HANR.[20] On the other hand, the total nitrogen content of HANR and fresh NR after incubation without urea in the presence of 1 wt % of SDS decreased to 0.028 wt % (S-DPNR) and 0.088 wt % (fresh S-DPNR), respectively; these values were higher than those of U-DPNR and fresh U-DPNR. It is thus quite important to incubate with urea for the preparation of DPNR.

A plot of the total nitrogen content versus the deproteinization time of HANR and fresh NR is shown in Figure 2. The total nitrogen content of HANR and fresh NR decreased drastically to 0.022 and 0.005 wt %, respectively, after 10 min. The total nitrogen content of HANR and fresh NR was observed to decrease further to 0.020 and 0.004 wt %, respectively, after incubation for 1 h. This indicates that almost all proteins are removed by incubation with urea for at least 10 min.

The amount of urea required for optimum deproteinization of NR latex was investigated, and the results are summarized in Table II. The total nitrogen content was found to be lowest when 0.1 wt %

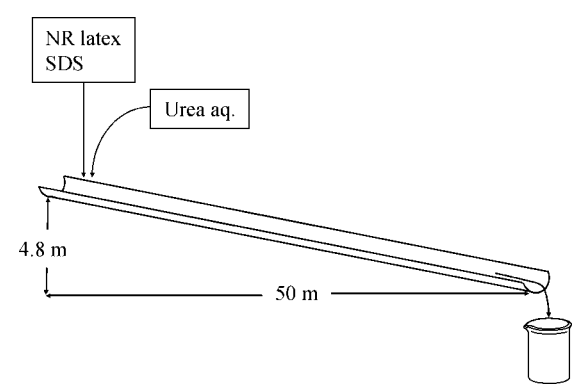

Figure 4 Semicircular channel for continuous incubation.

TABLE III
Total Nitrogen Content of DPNR Through Continuous Incubation

Specimen	Concentration of urea (wt %)	Total nitrogen content (wt %)
U-DPNR	0.1	0.023
U-DPNR	0.9	0.032
U-DPNR	8.0	0.032

urea was used. The higher total nitrogen content of 0.006 wt % at 0.04 wt % urea may be due to a lower amount of urea interacting with the proteins present in the rubber. In contrast, the higher total nitrogen content with a higher amount of urea may be due to the presence of an increased amount of residual urea because the total nitrogen content was found to increase linearly as the amount of urea increased.

Figure 3 shows Fourier transform infrared spectra of fresh U-DPNR, in which peak height, A, was normalized in comparison with a height of reference peak at 1660 cm^{-1}, A0, which was identified to a stretching vibration of carbon-carbon bond. The peak at 3320 cm^{-1} was assigned to monopeptides or dipeptides, as reported in the previous study.[21,22] On the other hand, the intensity of a small peak identified as the residual urea at 3450 cm^{-1} increased as the amount of urea increased. Thus, it is difficult to remove the residual urea from DPNR prepared by the incubation of NR latex with a higher amount of urea, even after the latex is centrifuged several times. This demonstrates that the total nitrogen content depends on not only the amounts of proteins and peptides but also the amount of the residual urea.

To scale up the DPNR preparation, it is necessary to design a continuous process for the incubation of NR latex with urea. The continuous process may be performed by the mixture of the latex and urea flowing in the presence of SDS for 10 min through a

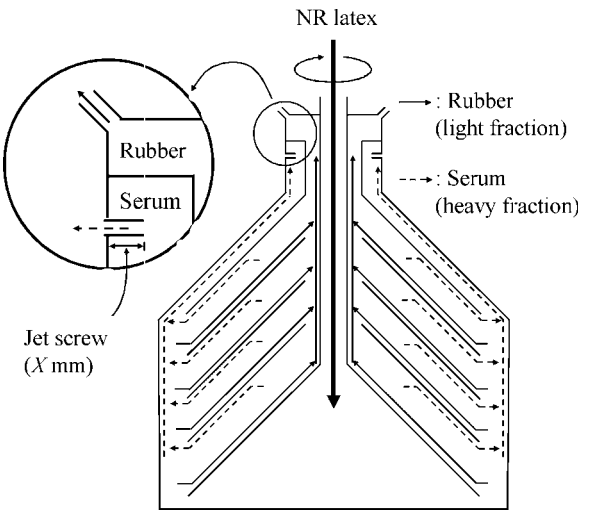

Figure 5 Bowl body of the continuous centrifuge.

TABLE IV
Total Nitrogen Content of U-DPNR Through Continuous Centrifugation

Specimen	Jet screw (mm)	Frequency of centrifugation	Total nitrogen content (wt %)
U-DPNR	11	1	0.057
U-DPNR	11	2	0.036
U-DPNR	9	1	0.050
U-DPNR	9	2	0.023

semicircular channel 50.4 m in length and 150 mm in diameter, as shown in Figure 4. Table III shows the total nitrogen content of U-DPNR prepared through continuous incubation followed by washing three times with a batch-type centrifuge. The total nitrogen content of U-DPNR prepared through continuous incubation of HANR latex in the presence of 8.0 wt % urea followed by centrifugation was 0.032 wt %, whereas it was reduced to 0.023 wt % after continuous incubation with 0.1 wt % urea. Because the total nitrogen content of U-DPNR prepared through continuous incubation with 0.1 wt % urea was quite similar to that prepared through batch incubation, mixing HANR latex with urea was proved to be completed through a semicircular channel for 10 min. This implies that continuous incubation may be applicable to a continuous process to remove the proteins from NR latex rapidly and efficiently.

To establish the continuous process of deproteinization of NR latex, we investigated the continuous centrifugation. Figure 5 shows the interior of a continuous centrifuge, that is, bowl body. The latex is fed from the top of the bowl body by gravity. The high-speed rotation of the bowl body results in a sufficient centrifugal force to separate the latex into two fractions, that is, a rubber fraction and a serum fraction. The serum fraction flows to the periphery of the bowl body, passes along the periphery, and is discharged through a jet screw. The rubber fraction is displaced toward the center of the bowl body and overflows through holes at top.

Table IV shows the total nitrogen content of U-DPNR prepared with a continuous centrifuge. The total nitrogen content of HANR decreased to 0.050 wt % after the first centrifugation with a 9-mm jet screw, which was lower than that with an 11-mm jet screw. A further decrease in the total nitrogen content was observed to 0.023 wt % after the second centrifugation with a 9-mm jet screw, which was similar to that prepared by the batch process of deproteinization. These results indicate that the total nitrogen content of U-DPNR depends on not only the conditions of continuous incubation but also the conditions of continuous centrifugation, such as the frequency and length of the jet screw. Under suitable conditions, subsequent work to establish the continuous process of deproteinization is in progress.

CONCLUSIONS

The removal of proteins from NR latex through a batch process was developed by incubation with urea in the presence of SDS for less than 10 min. For applications, continuous incubation and centrifugation were individually achieved by the use of a semicircular channel and a continuous centrifuge, respectively, to scale up DPNR preparation. The total nitrogen content of U-DPNR prepared through continuous incubation and centrifugation was quite similar to that prepared through a batch process, which was about 0.02 wt %. This study strongly indicates that a continuous process of deproteinization of NR latex with urea may be established by a combination of continuous incubation and centrifugation in the near future.

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