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SHORT COMMUNICATION

FTIR Studies on Amino Groups in Purified Hevea *Rubber*

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FTIR spectrum of natural rubber shows characteristic bands of attached nitrogeneous compounds at 3280 cm^{-1} and 1540 cm^{-1}. These bands diminish if the fresh field latex is treated with enzyme followed by washing in the presence of surfactant; a band at 3316 cm^{-1} is noticeable after the treatment. The band at 3320 cm^{-1} that remains after the treatments suggests the presence of residual amino acids bonded to the rubber molecule.

Natural rubber contains various types of naturally occurring non-rubber materials, which are either tenaciously held or bonded to the main-chain molecule. While studies on the non-rubber constituents in latex have been well documented, the bonded non-rubbers on the polymer chain have attracted less attention. This is probably due to their presence at very low concentration level and consequently the difficulty in characterising them.

Proteins and amino acids in natural rubber have been thought to be physically held by the polymer. Although they have been assumed to be responsible for the branching and crosslinking of natural rubber[1], their direct bonding to the polymer has not been demonstrated.

This paper reports a new finding on the presence of residual amino groups in highly purified *Hevea* rubber.

MATERIALS AND METHODS

Alcalase 2.0 *M* was obtained from Novo Industries, Japan and latex concentrate, 60.2% d.r.c., from Soctex, Malaysia.

Method 1

15 ml of latex concentrate was diluted to 200 ml by distilled water and stabilised with 0.12% w/v sodium naphtenate. The pH was adjusted to 9.2 by the addition of sodium dihydrogen phosphate. 0.78 g of alcalase 2.0 *M*, dispersed in 10 ml of distilled water, was added to the diluted latex. The pH of the solution was again adjusted to 9.2 accordingly. The mixture was allowed to stand for 24 h at 37°C.

Procedure A. The reacted latex was coagulated by the addition of 2% v/v phosphoric acid and washed extensively with distilled water. The rubber was dried under vacuum and extracted with acetone for 16 h. It was then redissolved in toluene at 1% w/v, and centrifuged for 30 min at 11 000 r.p.m. The clear solution was separated and the rubber precipitated into excess methanol. The rubber was dried under vacuum at room temperature[2]. The control sample was prepared by repeating the same procedure, omitting the alcalase treatment.

Procedure B. Upon completion of the enzymatic treatment, 1% w/v Triton X-100

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was added into the mixture and centrifuged for 30 min at 11 000 r.p.m. The cream fraction was redispersed into 200 ml of distilled water containing 1% w/v Triton X-100 and recentrifuged for 30 min. The process was repeated three times and the rubber was isolated by the addition of calcium chloride. Centrifugation may be necessary to assist coagulation. The coagulated rubber was treated as described above.

Procedure C. Further purification of deproteinised natural rubber was performed with ethanolic hydrochloric acid. Thus 0.5 g of rubber obtained from *Procedure B* above was dissolved in 100 ml of nitrogen-purged toluene containing 0.5 ml acetic acid for 24 h in the dark. Approximately 50 ml, 1 *M* nitrogen-purged HCl containing 10% ethanol was added into the solution and shaken for 20 min in a separating funnel. The rubber solution was separated by means of centrifugation and the isolated rubber solution was extracted with fresh ethanolic HCl. This was repeated four times. Finally, the excess acid was removed by washing with nitrogen-purged distilled water. The rubber was isolated by precipitating it into excess methanol. It was further purified by reprecipitation and dried under high vacuum.

Method 2

Latex concentrate was diluted 15 times with distilled water and stabilised with 1% w/v Triton X-100. The latex was centrifuged for 30 min at 11 000 r.p.m. and the cream was dispersed in a same volume of distilled water containing 1% w/v Trition X-100 and the centrifugation process was repeated. The procedure was repeated three times. The rubber was isolated with calcium chloride and extracted with acetone as described earlier.

Nitrogen contents in the *Hevea* rubber samples were analysed according to the procedure described in the RRIM test method[3]. Natural rubber was cast on KBr disk and transmittance was measured by a JASCO 5300 fourier transform infrared spectrometer for 300 scans with auto gain.

RESULTS AND DISCUSSION

Table 1 shows the nitrogen content of control sample which reflects the normal level of proteineous compounds in commercial latex concentrate. Purification by *Method 1, Procedure B* and *Method 1, Procedure C* gives much lower nitrogen content as compared to *Method I, Procedure A*. This is not totally suprising as in *Procedure A* the cleaved protein can be coprecipitated with natural rubber by phosphoric acid. Subsequent centrifugation after the deproteinisation process reduces nitrogen content to 0.01%. Alcoholic acid treatment as outlined, further reduced the nitrogen content to 0.009%.

FTIR study has revealed that non-purified rubber shows weak transmittance

TABLE 1. NITROGEN CONTENT OF PURIFIED NATURAL RUBBER

Sample	Nitrogen (%)	FTIR band (cm^{-1})
Control	0.16	3280, 1540
Method 1, Procedure A	0.05	3316, 3280, 1540
Method 1, Procedure B	0.01	3320
Method 1, Procedure C	0.009	3320
Method 2	0.04	3316, 3280, 1540

bands at 3280 cm^{-1} and 1540 cm^{-1} which are characteristic vibrations of >N–H and >N–C=0 respectively[4]. Upon successive surfactant washing, or enzymatic deproteinsation followed by acid coagulation, the intensities of these bands were significantly reduced. Concurrently, a band around 3316 cm^{-1} was found to be present. Further

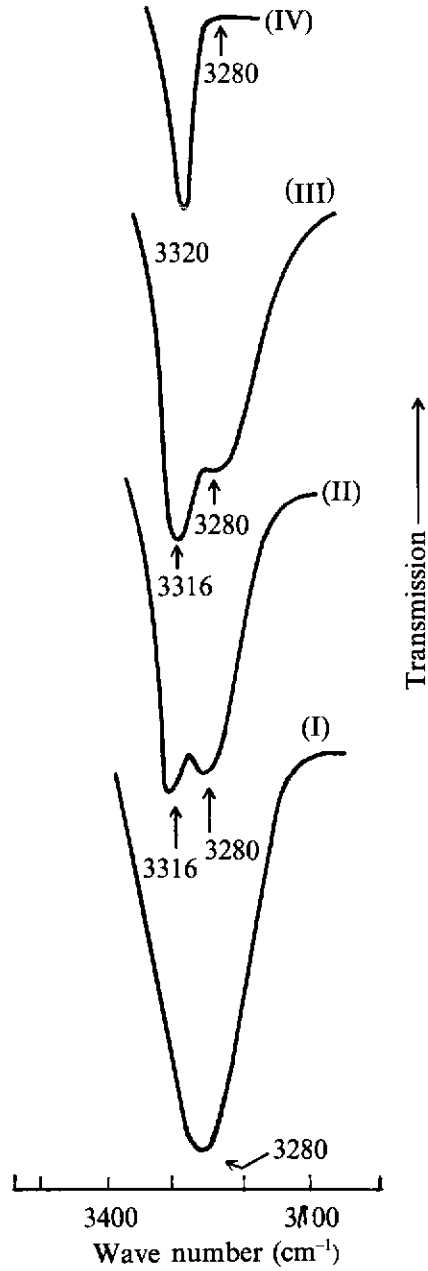

Figure 1. FTIR Spectra of purified Hevea *rubber.*
(I) Control, (II) Method 1, Procedure A, (III) Method 2, (IV) Methods 1, Procedure B and 1, Procedure C.

purification *via* successive surfactant washing after enzymatic treatment shows no detectable transmittance at 3280 cm^{-1} and 1540 cm^{-1} The band at 3320 cm^{-1}, however, remained present even after acid extraction as shown in *Figure 1*

Recent vibrational spectroscopic studies on a series of short chain peptides by Naumann *et al*[5] showed that >N–H in terminal amino acid of peptide, demonstrated by dipeptide and tripeptide, has a vibrational frequency at 3315 – 3320 cm^{-1} As the number of repeating units of peptide increases, the relative intensity of the inner >N–H units of the peptide linkages which have a vibrating frequency at 3280 cm^{-1}, increases while that of the terminal units reduce accordingly, as shown by tetrapeptide and pentapeptide In addition, in the associated state (bonded) and dissociated state, the >N–H peaks appeared at about 3313 cm^{-1} and 3390 cm^{-1} respectively[6] Therefore, the presence of >N–H band at 3320 cm^{-1} in purified natural rubber even after alcoholic acid treatment suggests that some proteins or amino acids are bonded to the natural rubber molecule This is not totally unexpected since proteins and amino acids have been demonstrated to react with the abnormal groups of natural rubber giving rise to the storage hardening phenomenon of natural rubber[7,8]

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