Structural Characterisation of the Small Rubber Particles in Fresh Hevea Latex

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The rubbers from small and large rubber particles were separated from the serum and cream phases of centrifuged fresh Hevea latex, respectively. The small rubber particles (SRP) consist of rubber molecules showing a unimodal molecular weight distribution (MWD) with a peak-top value falling between the two peak values of the bimodal MWD observed in rubbers from the large rubber particles (LRP). The rubber in LRP from mature trees and SRP from seedlings are polyisoprene molecules terminated by phospholipids comprising branching points, while that in SRP are presumed to be linear molecules containing no phospholipid terminal. Comparative data of MW and structure between the SRP obtained from the serum phase of fresh latex and those of the SRP obtained from seedling latex are reported. The mechanism controlling MW in connection with the relationship among MW, branching, gel and particle size.

Extensive investigations have been carried out on the types of rubber particles from centrifuged fresh natural rubber (NR) latex^{1,2}, as well as on the molecular weight (MW) and molecular weight distribution (MWD)^{3,4} of the rubber molecules they contain. Gomez *et al.*¹ tried to elucidate the physical structure of rubber particles of different particle sizes. Chen⁴ reported that the mean particle diameters, measured by using a soap adsorption method, have no relationship with the MW of rubbers from several clones². However, Subramaniam³ and Yeang *et al.*⁵ observed that the small rubber particles contain rubbers of very high MW^{1,3}. Thus, the rubber particle size is believed to be an important parameter related to the biosynthesis mechanism(s) controlling MW in *Hevea* rubber.

Although there have been many investigations of MW and rubber particle size, there has been no report on the structure of rubber molecules from different particle sizes so far. It is important to establish the relationship between the molecular structure of rubbers and the particle size, because these are related to the biosynthesis mechanism controlling MW in *Hevea* rubber. Reports of such studies are presented in this paper.

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EXPERIMENTAL

Material Preparation

Freshly tapped latex (FL-latex) from mature Hevea brasiliensis trees of RRIM 600 clonal origin was first centrifuged at 5000 g for 30 min to remove the bottom fraction, consisting mainly of non-rubber particles. The latex then was dispersed into 1% Triton X-100[®] solution and centrifuged at 10000 g for 1 h to separate the cream rubber from the serum phases. The serum phase, containing about 7% dry rubber content (DRC), was subjected to further centrifugation at 25 000 g for 2 h to collect the residual rubber (serum rubber). Each rubber phase was coagulated by acetone and purified by reprecipitation from toluene solution with methanol $(3\times)$, and dried in vacuo. Acetone extraction of the rubber samples was carried out under a nitrogen atmosphere for 24 h in a Soxhlet apparatus to remove some of the non-rubber components. such as the free fatty acid. Transesterification of the rubber in toluene solution was carried out by the method described previously, using freshly prepared sodium methoxide (NaOCH₃)⁶, to destroy branches and crosslinks attributable to the presence of fatty acid esters of polyhydric alcohols. All the solvents were supplied by BDH, Thailand (Bang Trading 1992 Co., AN RI Ltd.).

Freshly tapped latex from Hevea trees of different ages but the same clone was also subjected to particle size and MW analyses.

Molecular Weight and Particle Size Determination

The MW and MWD of the purified rubber were determined by gel-permeation chromatography (GPC) using two columns connected in series, packed with styrene-divinylbenzene

copolymers, having exclusion limits of 2.0×10^7 and 5.0×10^3 . Measurements were made using tetrahydrofuran (THF) as an eluent with a flow rate of 0.5 ml/min at 35°C, monitored with a TOSOH LS-8000 with refractive index (RI) and low-angle-laser light-scattering (LALLS) detectors. Commercially obtained standard polystyrenes were used for column calibration. Absolute number-average mole-cular weight $(\overline{M}n)$ of the sample was obtained by osmometry using a Wescan Membrane Osmometer. Measurements were made in toluene solution in the concentration range $1.5 \text{ g/dm}^3 - 5 \text{ g/dm}^3$ at 35.0°C. The particle size analysis was done using a Horiba LA-910 particle size distribution analyser (small volume module).

Gel, Ester and Nitrogen Content Determinations

The purified rubber was dissolved in dried toluene at a concentration of 0.2% (w/v) and kept in the dark without agitation for one week at room temperature to attain equilibrium, followed by centrifugation at 11 000 g for 40 min to separate the gel fraction. The isolated gel fraction was dried in vacuo at room temperature. The gel content was calculated as the weight ratio of the gel fraction to the original rubber. Quantitative analysis of the ester groups was done by FTIR spectroscopy, using a calibration curve obtained from a mixture of synthetic cis-1, 4-polyisoprene and methyl stearate⁷. The nitrogen content was analysed using a Yanaco CHN Corder MT-5 Elemental Analyzer.

Structural Analysis

The ¹³C-NMR measurements were carried out on CDCl₃ solutions of rubber with tetramethylsilane (TMS) as an internal standard using a JEOL ALPHA-500 NMR spectrometer, operating at 125 MHz with a pulse repetition time of 7 sec at 50°C.

RESULTS AND DISCUSSION

Figure 1 shows a typical particle size distribution for whole latex, cream and serum fractions from fresh latex. The rubber particles in the cream phase show a size distribution ranging from 0.1 μ m to 3.0 μ m, with a mean diameter of 1.03 μ m. On the other hand, the size distribution of the rubber particles in the serum phase is seen to be in a range of 0.05 μ m to 0.3 μ m, with a mean diameter of 0.13 μ m. The total latex had a similar particle size distribution to that of the cream phase. The peak of the SRP distribution below 0.2 μ m is not clear in the distribution for the total latex, due to its low rubber content, *i.e.*, less than 7% by weight.

Figure 2 shows the MWD of the purified cream and serum rubbers. The cream rubber

shows a typical bimodal MWD, with peaks at MW of 2.0×10^6 g/mol and 1.2×10^5 g/mol. It is obvious that the MWD of serum rubber is a unimodal, with a peak-top value centered at 1.0×10^6 g/mol and falling between the values of the high and low MW peaks in the MWD of the cream rubber.

The MW characteristics of these rubbers are given in *Table 1*. It is remarkable that the serum rubber shows $\overline{M}_{n,osmo}$ value higher than that of the cream rubber. However, no significant difference in the $\overline{M}_{w,LS}$ values of the rubbers was observed. These results coincide with those reported by Subramaniam³ and Yeang, *et al.*⁵

Table 2 shows the ester, nitrogen and gel contents of the rubbers from the cream and serum phases after purification by reprecipita-



Figure 1. Particle-size distributions of the rubbers from fresh latex.



Figure 2. Molecular-weight distributions of the rubbers from fresh latex.

TABLE 1. MOLECULAR-WEIGHT CHARACTERISTICS OF RUBBERS F	ROM
FRESH LATEX OF DIFFERENT PARTICLE SIZE	

Sample	Mean particle size (µm)	10 ⁻⁶ M _{w,LS} (g/mol)	$\overline{M}_w / \overline{M}_n$	10 ⁻⁵ M _{n,osmo} (g/mol)
Original rubber	1.18	1.9	9.2	(Not observed)
Cream rubber	1.03	2.5	10.5	5.5
		(2.0)	(10.3)	(3.2)
Serum rubber	0.13	1.0	2.6	6.8
		(0.9)	(2.5)	(6.4)

The values in parentheses are the data for rubbers after transesterification.

tion and acetone extraction. The average ester content of the purified cream and serum rubbers was 10.2 mmol/kg and 0.1 mmol/kg rubber, respectively. It has been shown that the terminating-end of the rubber molecule is functionalised by phospholipid, containing 1-2 long-chain fatty acid esters⁷. Based on this finding, the ester content is regarded as an

Sample	Ester content (mmol/kg)	Nitrogen content (% w/w)	Gel content (% w/w)
Original rubber	10 2	0 25	3 5
Cream rubber	81	0 22	71
	(0)	(0 21)	(0)
Serum rubber	0 1	0 02	13
	(0)	(0 02)	(0)

TABLE 2ESTER, NITROGEN AND GEL CONTENTS OF PURIFIED
CREAM AND SERUM RUBBERS

The values in parentheses are the data of rubbers after transesterification

index of fatty acid molecules per rubber chain The ester content observed in the cream and serum rubbers corresponds to about 2 and 0 03 molecules per rubber chain, respectively This result implies that the rubber molecules in SRP are not terminated by phospholipid groups as could be detected in the LRP molecules

The mitrogen content of the purified cream and serum rubbers was 0 22% and 0 02%, respectively Before purification, the mitrogen content was 0 41% and 0 83%, for the cream and serum rubbers, respectively It is known that the serum fraction contains many mitrogenous compounds derived mainly from enzymes used in rubber biosynthesis, *etc* ⁸ However, after purification of the rubber as described in the Experimental section, the residual mitrogen content of 0 02% in the serum rubber is expected to represent the mitrogenous compounds attached to rubber molecules This value corresponds to those observed in highly deproteimised natural rubber prepared by enzymatic deproteimisation⁶

It is well known that the gel content in rubber obtained from fresh latex is low, usually negligible However, this gel content increases up to about 30% with increasing storage period of the

latex in the presence of ammonia (Figure 3) The LRP rubber, which is known as a highammonia preserved latex, also shows the same tendency, increasing in gel content up to 60% In contrast, the gel content in serum rubber increases insignificantly during storage in ammonia (Figure 3) We have confirmed that transesterification can destroy crosslinks and branches which were formed from ester group9 The content of gel and ester groups reduced to zero after transesterification of both rubbers from SRP and LRP, while the nitrogen content did not decrease significantly (Table 2) Furthermore, in the case of cream rubber or LRP, the \overline{M}_{wLS} and $\overline{M}_{n \text{ osmo}}$ values also decreased by about 20% and 35%, respectively (Table 1) However, no significant change of \overline{M}_{wLS} and $\overline{M}_{n \text{ osmo}}$ was observed for the serum rubber as a result of transesterification (Table 1) This suggests that the cream rubber consists of branched and crosslinked molecules as mentioned above, while the serum rubber is composed of linear molecules

The low content of phospholipid linked to the serum rubber molecule was confirmed by 13 C-NMR, as shown in *Figure 4* The purified serum rubber shows almost no 13 C-NMR signals at



Figure 3. Changes in gel content of rubber fractions obtained from (A) fresh latex; (B) high-ammonia preserved latex and (C) serum phase, during storage in 0.4% ammonia for 2 months.



Figure 4. ¹³C-NMR spectra of rubbers from (A) cream and (B) serum phases.

174.2, 34.4, 29.7 and 14.0 p.p.m., these being characteristic signals of the carboxylic-carbon (-CH₂- \underline{CO}_2 -), terminal methylene- (- \underline{CH}_2 -CO₂-), methylene- (-(\underline{CH}_2)-) and methyl-carbon (\underline{CH}_3 -) atoms in the long-chain fatty acid groups, respectively. These signals can be seen in the spectrum of the purified cream rubber. A small signal distinguishable from noise was observed at 62.04 p.p.m. in the spectrum of the serum rubber. It is assigned to the C-4 methylenecarbon of the terminal isoprene unit linked to a mono- or di-phosphate group, but not phospholipid¹⁰.

From a biochemical study, Archer, *et al.*¹¹ reported that the SRP separated from fresh latex is more effective in the incorporation of an exogenous isopentenyl diphosphate (IDP) than the LRP, based on estimation of the incorporation content per unit surface area of rubber. Later, McMullen and McSweeney¹² confirmed this result and showed the high activity of SRP to incorporate the exogenous IDP with a diminishing of the rubber particle sizes. These findings support the idea that SRP is composed of mainly active rubber molecules, which are ready for chain extension to form high MW rubbers.

Another idea to support this assumption is that the associated phospholipid membrane¹³ on the SRP surface is expected to be extremely thin due to the small ester content and the small size of SRP. In other words, the amount of phospholipid binding on to the membrane of SRP is insufficient to terminate the living rubber molecule. Thus, it will lead to the formation of high MW rubber chains in SRP.

We have characterised the latex from *Hevea* seedlings¹⁴. The latex from seedlings contained low MW rubber having average particle size of 0.1 μ m. The MWD of seedling rubber shows a unimodal distribution with a peak at 1×10^5 and

 $\overline{M}_{n,osmo} = 6 \times 10^4$ g/mol. The structure of this rubber is almost the same as that observed for the LRP, *i.e.*, the chain end is terminated with phospholipid. We also observed the very low activity of the seedling rubber to incorporate IDP to form higher MW rubber (data will be presented in a subsequent paper). It is interesting that although the particle-size distribution and average particle size of the rubber from seedling latex are almost the same as those of SRP from mature tree, the MW and structural characteristics are quite different. It is clear from Figure 5 that both the average MW and mean particle size of rubber in latex from seedlings increase with increase in rubber tree age. A constant value of MW and particle size was attained in latex from trees which were 6-7 years old.

It has been reported that there is no direct relationship between the particle size and MW of NR^3 . However, based on the present results, it appears that the particle size of rubber latex is an important factor influencing the MW and MWD of *Hevea* rubber.

CONCLUSION

SRP in the serum phase of *Hevea* latex has a mean diameter of 0.13 μ m. The rubber molecules in SRP are not terminated by phospholipid as is the case for LRP and rubber from seedlings. The $\overline{M}_{n,osmo}$ value of SRP is higher than that of LRP, but there is no significant difference in the $\overline{M}_{w,LS}$ values for both rubbers. It was observed that the particle size distribution and average size of rubber particles from seedling latex are almost the same as those of SRP from mature tree. However, the $\overline{M}_{n,osmo}$ of the seedling rubber is lower than that of SRP by a factor of about ten. Thus, it can be presumed that the particle size of rubber latex is an important parameter influencing the MW and MWD of *Hevea* rubber.



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Figure 5. Changes in mean particle size (——) and molecular weight (\overline{M}_w ---- and \overline{M}_n ----) of Hevea rubbers from several rubber tree ages.

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REFERENCES

 GOMEZ, J B. AND MOIR, G.F.J. (1979) The Ultracytology of Latex Vessels in Hevea brasiliensis. MRRDB Monograph No. 4, 18.

- SCHOON, T.G.F. AND VAN DE BIE, G.J. (1955) Particle Size Distribution in Brominated Hevea Latices. J. Polym. Sci., 16, 63.
- 3. SUBRAMANIAM, A. (1980) Molecular Weight and Molecular Weight Distribution of Natural Rubber. RRIM Technol. Bull. 4, 20.
- 4. CHEN, S.F. (1979) Adsorption of Sodium Dodecyl Sulphate on Natural Rubber Latex Particles and Determination of the Specific Surface Area of the Particles. *Proc. Int. Rubb. Conf. 1979, Venice*, 215.
- 5. YEANG, H.Y., ESAH, Y. AND SAMSIDAH, H. (1995) Characterisation of Zone 1 and Zone

2 Rubber Particles in *Hevea brasiliensis* Latex J Nat Rubb Res, **10**, 108–123

- 6 TANGPAKDEE, J AND TANAKA, Y (1997) Purification of Natural Rubber J Nat Rubb Res, **12(2)**, 112–119
- 7 TANGPAKDEE, J (1998) PhD Thesis on Engineering submitted to the Tokyo University of Agriculture and Technology, Japan
- 8 ARCHER, B L, AUDLEY, B G, McSWEENEY, G P AND TAN, C H (1969) Studies on Composition of Latex Serum and Bottom Fraction Particles J Rubb Res Inst Malaya, 21, 560–569
- 9 TANGPAKDEE, J AND TANAKA, Y (1998) Characterization of Sol and Gel in *Hevea* Natural Rubber *Rubb Chem Technol*, **70**, 1
- 10 LI, B G , SAKDAPIPANICH, J T , KAWAHARA, S AND TANAKA, Y Structural Characteriza-

tion of the Terminating Species of Polyprenyl Phosphate *Phytochemistry*, to be submitted

- 11 ARCHER, BL, AUDLEY, BG, COCKBAIN, EG AND McSWEENEY, GP (1963) The Biosynthesis of Rubber Incorporation of Mevalonate and Isopentenyl Pyrophosphate into Rubber by *Hevea brasiliensis* Latex Fractions *Biochem J*, 89, 565
- 12 McMULLEN, A I AND McSWEENEY, G P (1966) The Biosynthesis of Rubber Incorporation of Isopentenyl Pyrophosphate into Purified Rubber Particles by a Soluble Latex Serum Enzyme *Biochem J*, **101**, 42
- 13 HO, C C , SUBRAMANIAM, A AND YONG, W M (1976) The Lipids Associated with the Particles in *Hevea* Latex *Proc Int Rubb Conf* 1975 Kuala Lumpur, 2, 441–456
- 14 TANGPAKDEE, J, TANAKA, Y, WITITSU-WANNAKUL, R AND CHAREONTHI-PHAKORN, N (1996) Phytochemistry, 42, 353