SEC-MALS Study of Dynamic Structuring of Natural Rubber: Comparative Study of Two *Hevea brasiliensis* Genotypes

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Received 27 January 2011; accepted 14 June 2011
DOI 10.1002/app.35099
Published online 20 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The dynamic structuring of natural rubber (NR) was studied selecting two specific *Hevea brasiliensis* genotypes (RRIM600 and PB235) to prepare model samples. The mesostructure (macromolecular structure + aggregates or gel) of NR samples was studied by SEC-MALS. The NR samples were analyzed after (i) slow structuring (18 months' storage at room temperature) and (ii) fast structuring (stored for 24 h on P_2O_5 at 60°C). This study showed that the macromolecular structure, especially M_n, and the total gel rates were dramatically modified after fast structuring. For genotype RRIM600, the aggregates formed during fast structuring were essentially macroaggregates, whereas for genotype PB235 mostly microaggregates were formed. These results indicate that the dynamic structuring of NR is dependent on genotype. Depending on the genotype, for extreme conditions (fast structuring), it can be assumed there was percolation between elementary bricks, probably microaggregates, or no percolation. Although the mechanisms of dynamic structuring are quite complex and should be multifactors dependent, on the basis of our results, the degree of percolation seems to be partly dependent on the quantity of short polyisoprene chains initially present in the NR samples. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1570–1577, 2012

Key words: natural rubber; dynamic structuring; storage hardening; gel; SEC-MALS

INTRODUCTION

Two structural parameters are known to be of paramount importance for natural rubber properties compared with its synthetic counterpart. The first parameter is the quasi 100% *cis* configuration of the polyisoprene chains. The second is the associative structure generating aggregates of different sizes, usually called the gel phase. This gel phase is insoluble in common solvents of polyisoprene and is generated by links/interactions between non-isoprene compounds (proteins, lipids, etc.) and polyisoprene macromolecules.[1–5] The main difficulty in studying this associative structure lies in its dynamic character. Indeed, the associative structure of NR is not static but dynamic, changing over time (storage) because of the phenomenon specific to NR known as storage hardening. During this process, the NR structure evolves to produce gel which increases over the sample storage time.[6–8] Many studies have been undertaken to identify mechanisms and natural compounds of latex involved in NR hardening during storage,[9–16] but to our knowledge, no studies have been published about changes in NR macromolecular structure during storage. Several compounds of latex involved in crosslinking reactions have been proposed: amino acids,[9,11] inorganic ions (Mg^{2+}, Ca^{2+}),[13] proteins,[16–19] phospholipids.[15] These compounds react with so-called abnormal groups on the polyisoprene chains, such as carbonyl and aldehyde groups,[9,20] epoxide groups,[10,21] lactone,[22] and phospholipids,[15] to produce gel. Ngolemasango et al.[14] assumed that these abnormal groups were situated preferentially on short polyisoprene chains. The involvement of proteins in structuring the gel in NR has been proposed by several authors,[17–19] while others assumed that phospholipids are the key parameter[23]. It is also worth mentioning the recent results obtained by Intapun et al.[24] which showed

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Contract grant sponsors: The Commission of Higher Education, Thailand; The French Embassy Bangkok.

Journal of Applied Polymer Science, Vol. 124, 1570–1577 (2012)
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that the presence of microorganisms in the initial latex had a significant influence on gel formation in NR. The increase in gel in line with the quantity of microorganisms in the initial latex was enhanced with the coagulum "maturation," or storage time, under controlled environmental conditions. Ehabe et al.[25] showed the importance of this natural step before processing, coagulum maturation in the field, in the structure and certain properties of NR. All these published results show that the mechanisms of gel formation in NR can be somewhat diverse and complex.

Another important point when studying the mesostructure (macromolecular structure + aggregates or gel) of NR, it is that not all authors determined the gel rate by the same method and did not therefore measure the same thing. Many methods can be used to determine the quantity of gel in a polymer[26]. For NR, the gel rate has often been determined by gravimetry, after centrifugation, and is usually called the "macrogel" or "gel phase." Other names for this "centrifuge" gel in NR can be found in the literature ("true gel," etc.). Not all authors used the same solvent, and the latter has considerable importance for the gel rate determined.[14,27,28,29] A few authors used more complete methods able to determine also the microaggregates fraction, usually called "microgel,"[1,3,14,30,31] remaining in solution after centrifugation. In a THF solution of a NR sample (RSS type), Shiibashi[18] found 9% of gel after centrifugation (macrogel) and 49% additional gel after filtration of the centrifuged solution (microgel). The gel rate can also be determined by SEC after filtration of the solution to be analyzed[5,14,32]. The advantage of this method lies in the determination of the total gel rate and the macromolecular structure using the same solution in one spot. The porosity of the filters generally used (0.45 or 1 μm) has a considerable impact on the molar mass distribution (MMD) and the gel rate determined.[5] In addition, macrogel and microgel are often inversely correlated, the more macrogel in a sample the less microgel and *vice versa*,[3] which it is rather awkward when determining low macrogel content without quantifying microgel. It is also important to keep in mind that some of the aggregates are formed by physical links. Indeed, adding a small quantity of polar solvent (ethanol) to toluene or cyclohexane can decrease the macrogel rate.[4,27]

Kim et al.[5,33] analyzed NR samples with SEC-MALS and showed that the soluble part injected into a SEC system contained very few branched macromolecules, contrary to earlier published studies.[34–36] Kim et al.[5,33] showed that the soluble part of NR in solution in tetrahydrofuran (THF) was composed of a mixture of linear chains and assumed compact microaggregates ($R_g \approx$ 110–130 nm). They showed that, to more effectively ascertain the mesostructure of NR, a MALS detector needs to be used with SEC to avoid a misunderstanding of the structure, as is the case with most polymers.

This article presents a study by SEC-MALS undertaken to gain a clearer understanding of the dynamic structuring of the mesostructure in NR. On the basis of a previous study results[5,14] two model genotypes of *Hevea brasiliensis* were chosen, because of the importance of the native molar mass distribution of rubber (Fig. 1) in governing certain properties of NR[5,37]. Changes in the macromolecular structure of NR were studied before and after (i) slow structuring (18 months' storage at room temperature) and, (ii) fast structuring (stored for 24 h on P_2O_5 at 60°C).

EXPERIMENTAL

Materials

The NR samples used for this study were ribbed smoked sheet (RSS) from Thailand prepared from monoclonal latex. Two genotypes were used to prepare the RSS samples, PB235 with a quasi-unimodal elution profile, and RRIM600 with a bimodal elution profile (Fig. 1). The RSS were prepared as described by Rodphukdeekul et al.[38] The films for analysis of native macromolecular structure (Fig. 1) were prepared from fresh latex collected 6 h after tapping. One mL of latex was poured onto a glass plate (20 × 20 cm) and spread out, then dried with a nonheating hair-dryer for 10 min. The film was washed for 30 min in distilled water at 50°C and dried again.

Fast structuring conditions

The samples were homogenized according to ISO 1795 and a thin sheet was prepared according to ISO1795 and ISO2393. The homogenized sample was then passed three times through the gap of a two-roll laboratory mill at room temperature. The gap was adjusted so that the final thickness was between 1.6 and 1.8 mm. The sheeted rubber was doubled after the final pass and 24 pellets were cut with a Wallace punch in the double sheet obtained. The pellets were between 3.2 and 3.6 mm thick, with a diameter of about 13 mm. The pellets were divided into two sets; one set was used for fast structuring of the samples and the other set as a control. Fast structuring of the samples was achieved by storing them in a jar containing phosphorus pentoxide (P_2O_5) for 24 h in an oven at 60°C.

Wallace plasticity (P_0) measurement

A Wallace plastimeter, a specific pseudorheological tool for NR,[39] was used to measure plasticity, in accordance with standard ISO2007. A homogenized pellet is compressed between two circular platens

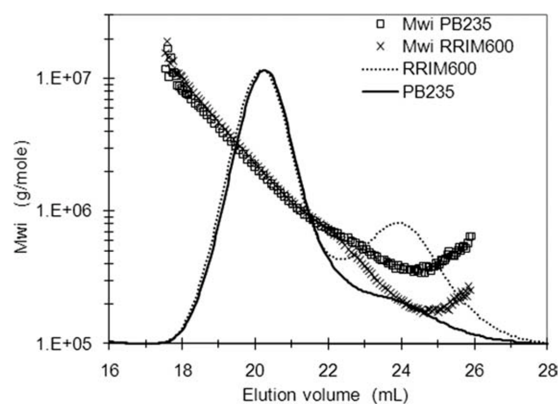

Figure 1 Chromatograms showing the refractometer signal as a function of elution volume for films made with fresh latex from genotype PB235 and genotype RRIM600. This elution profile is called native elution profiles because it is as close as possible to what flows from the tree (see Experimental section).

which are maintained at a temperature of 100°C. The sample is conditioned for 15 s at a thickness of 1 mm. A compressive force of 100*N* is then applied for 15 s. The final thickness of the test piece multiplied by 100 and expressed in units of 0.01 mm is the Wallace plasticity (P_0). The higher the Wallace plasticity the harder the NR sample is. The higher the increase in Wallace plasticity along storage, the higher the propensity of NR samples to undergo dynamic structuring.

SEC-MALS analysis

The samples (30 ± 5 mg), from NR pellets, were dissolved in pure tetrahydrofuran (THF, 30 mL, HPLC grade, VWR France) stabilized with 2,6-di-*tert*-butyl-4-methylphenol (BHT). Three solutions were prepared for each sample and each solution was injected once into a SEC-MALS system. After storing for 7 days in the dark at 30°C (during the storage time, the solutions were stirred 1 h/day), the solutions were filtered (Acrodisc 1 μm, glass fiber, Pall France) and injected into a SEC-MALS system. As the exact initial concentration of the sample solutions was known and the injected quantity was determined after filtration and elution, it was possible to determine the recovery rate and thus the insoluble part or total gel rate. The SEC equipment consisted of an online degasser (Elite™, Alltech), a Waters 515 pump, a refractive index detector (Waters 2410) and a multi-angle light scattering detector (Dawn DSP, Wyatt Technology). The columns, maintained at 45°C, were two Waters HMW6E (20 μm, 300 mm × 7.8 mm I.D.) plus one PLgel (Polymer Laboratories) Mixed-A (20 μm, 300 mm × 7.8 mm I.D.) with a guard column. The mobile phase was stabilized THF at a flow rate of 0.65 mL/min; the injected volume was 150 μL. All diode detectors at all 18 angles in the MALS instrument were normalized using a THF solution of low polydisperse polystyrene standard (M_w = 30.3 kg/mol, Wyatt technology). The same solution was used to determine the interconnection volume between the two detectors (0.235 mL). The basic theory of determining the weight-average molar mass and radius of gyration for a dilute solution of a macromolecule is well known and has already been described in numerous papers in the literature.[40,41] The weight-average molar masses and radius of gyration at each slice of the chromatogram were calculated using the Berry method for extrapolation in ASTRA software version 5.3.1 (Wyatt technology). The order of polynomial fit used with the Berry method was two. Twelve angles, from angle 5 (38.8°) to angle 16 (138.8°), were used for calculation. The integration limits used for calculation were from 27–27.5 to 40–41 min according the sample. The differential refractive index increment (d*n*/d*c*) value at 633 nm was 0.130 mL/g.[42]

RESULTS AND DISCUSSION

Influence of fast structuring on the mesostructure of fresh NR samples

Figure 2 shows the elution profiles (DRI signal) and the evolution of molar masses as a function of the elution volumes for the fresh NR samples made from genotypes PB235 and RRIM600, before and after fast structuring (60°C for 24 h on P_2O_5). For both genotypes, the MMD of fresh NR samples before fast structuring on P_2O_5 remained very similar to that of the native NR (Fig. 1): unimodal and bimodal elution profiles for PB235 and RRIM600, respectively. After fast structuring, the Wallace plasticity (P_0) for PB235 dramatically increased from 48 to 79.5 (Table I) and the insoluble part or gel rate followed the same trend, increasing from 24 to 52% (Table I). Fast structuring promoted interactions between polyisoprene chains as confirmed by the increase in P_0 and total gel rate, as reported in previous studies.[6,14] However, despite the large changes in P_0 and total gel rate, very little change was observed in the molar mass distribution of genotype PB235 [(Fig. 2(a)]. Despite quite the same elution profiles before and after fast structuring on P_2O_5, the weight-average molar mass (M_w), z-average molar mass (M_z) and especially the number-average molar mass (M_n) exhibited significant changes. M_n increased significantly from 990 to 1410 kg/mol (Table I). This increase in M_n, was mainly due to more pronounced abnormal elution after fast structuring [Figs. 2(a) and 3(a)]. Kim et al. [5] showed that abnormal elution is because of delayed, supposed

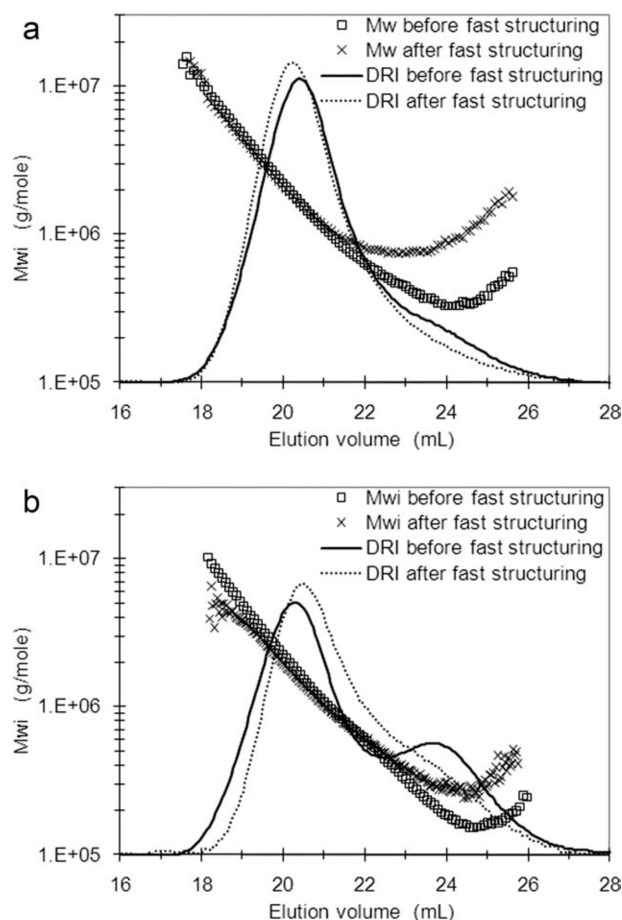

Figure 2 Chromatograms showing the refractometer signal and molar masses (M_{wi}) as a function of elution volume for fresh NR samples before and after fast structuring on P_2O_5 (a) genotype PB235 and (b) genotype RRIM600

compact, microaggregates during elution in SEC columns. These delayed microaggregates elute with short chains at high elution volumes, thus causing an increase in the M_{wi} and R_{gi} determined at high elution volumes.[5] Several mechanisms were proposed to explain the retardation of microaggregates: (i) an anchoring of branched macromolecules, by Podzimek et al.[43]; (ii) a phenomenon called "slalom chromatography" by Guillaume and coworkers[44] or (iii) chemical adsorption on the columns packing.[5] The phenomenon involved in delaying natural rubber microaggregates is still unclear for the moment. It can be noticed that M_z, unlike M_w and M_n, decreased after fast structuring (Table I). For samples from genotype RRIM600, fast structuring had a more important influence on P_0 and the gel rate than for samples from genotype PB235 (Table I). P_0 increased from 36 to 98 and the gel rate from 24.5 to 77% (Table I). In contrast with PB235, the elution profiles of samples from genotype RRIM600 drastically changed after fast structuring [Fig. 2(b)]. Indeed, the bimodal elution profiles before fast structuring became quasi-unimodal afterwards. A significant quantity of short and long polyisoprene chains disappeared, being most probably retained in the gel phase. It was difficult at this step to know if all these chains were reactive chains and participated in gel structuring (active trapping), or if most of them were retained without involvement in structuring (passive trapping). As a consequence of this elution profiles change, M_n, M_w and M_z also changed significantly after fast structuring (Table I). Although M_w and M_z decreased, M_n increased from

TABLE I
Wallace Plasticity (P_0), Total Gel Rate, Number-Average Molar Mass (M_n), Weight-Average Molar Mass (M_w), z-Average Molar Mass (M_z), and Radius of Gyration (R_g) of NR Samples from Genotypes PB235 and RRIM600 Before and After Slow and/or Fast Structuring

	PB235		RRIM600	
Parameters	Before fast structuring (a)	After fast structuring	Before fast structuring	After fast structuring
Fresh samples				
P_0 (1/100 mm)	48 (±1.0)	79.5 (±0.8)	36 (±8.2)	98 (±3.8)
Total Gel (%)	24 (±2.0)	52 (±2.7)	24.5 (±0.1)	77 (±0.7)
M_n (kg/mole)	990 (±25)	1410 (±36)	530 (±8)	700 (±45)
M_w (kg/mole)	1780 (±23)	1910 (±22)	1590 (±5)	1220 (±6)
M_z (kg/mole)	3120 (±47)	2730 (±27)	3330 (±25)	1960 (±45)
R_g (nm)[b]	110.7 (±0.1)	107.0 (±1.6)	116.0 (±0.8)	89.0(±2.3)
Samples stored for 18 months (slow structuring)[c]				
P_0 (1/100 mm)	55 (±0.8)	76 (±1.5)	45 (±0.9)	96 (±0.4)
Total Gel (%)	32 (±1.0)	43 (±1.5)	33 (±1.3)	64 (±1.0)
M_n (kg/mole)	1176 (±29)	1390 (±30)	870 (±20)	1200 (±110)
M_w (kg/mole)	1720 (±29)	1830 (±44)	1700 (±25)	1740 (±89)
M_z (kg/mole)	2640 (±80)	2580 (±67)	3000 (±170)	2530 (±86)
R_g (nm)[b]	100.8 (1.1)	100.7 (±1.3)	107 (±0.8)	99 (±2.2)

The numbers in brackets are the standard deviation obtained on three replicates.
[a]Fast structuring: samples were stored for 24 hours on P_2O_5 at 60°C.
[b] z average (R_z) given by Astra software.
[c] Slow structuring: samples were stored at room temperature in the laboratory for 18 months

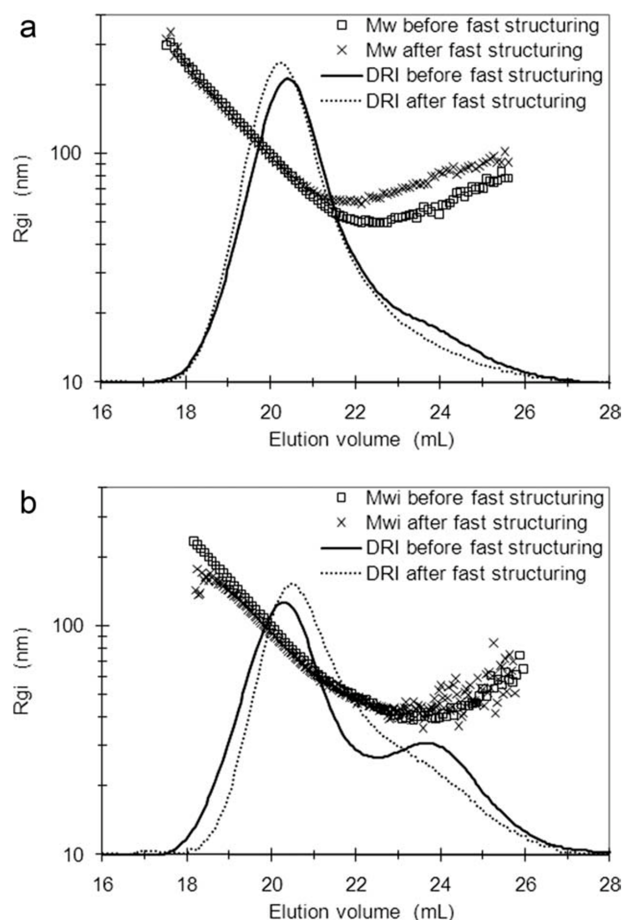

Figure 3 Chromatograms showing the refractometer signal and radius of gyration (R_{gi}) as a function of elution volume for fresh NR samples before and after fast structuring on P_2O_5 (a) genotype PB235 and (b) genotype RRIM600

530 to 700 kg/mol. For genotype RRIM600, it was difficult to interpret the M_{wi} profile in the high elution volume, or the zone of short chains, because of the mixture of short chains and delayed microaggregates. Nevertheless, for the samples of this genotype after fast structuring, the elution abnormality was not so pronounced as for genotype PB235 (Fig. 2). The increase in M_n in the case of genotype RRIM600 samples was most probably due mainly to the disappearance of short chains [Fig. 2(b)]. It has been noticed that, whatever the genotype, the conformation plots ($R_{gi} = f(M_{wi})$ exhibited no significant difference, which means there was no significant apparent difference in terms of branching or structure for the soluble material whatever the treatment (results not shown).

From a qualitative point of view, after fast structuring on P_2O_5, although PB235 samples contained 52% of gel, there was no visible gel in the flask of PB235 samples after 7 days of solubilization in THF (Fig. 4). On the other hand, the sample from genotype RRIM600 exhibited a very visible gel. This means that fast structuring particularly induced microaggregates in the case of samples from genotype PB235 and macroaggregates (macrogel) for genotype RRIM600. This macrogel could have entrapped nonreactive polyisoprene chains. In other words, for samples from genotype RRIM600 there was percolation between the reactive entities to generate macrogel but not for genotype PB235, generating only microaggregates, which is in accordance with the higher elution abnormality in SEC-MALS. On the basis of sol–gel transition in polymer solutions,[45] it can be assumed that the microaggregates could be formed and interact together to form meso and macroaggregates. A mechanism was proposed by Tanaka's group[4] to explain gel formation in natural rubber. This mechanism postulated that the two ends of the polyisoprene chains had reactive groups, a protein at the ω-terminal and a phospholipid at the α-terminal. On this basis, and taking into account the results of Kim et al.[5] regarding the compactness of microaggregates, it can be imagined that the core of the microaggregates should be a protein and the shell formed by polyisoprene chains with a phospholipid as the end group. But, it is worthy to mention that Carretero et al.[23] proposed the contrary: a core-shell structure with phospholipids in the core. Whatever the reality, the very interesting result in our study was the different behavior observed for the samples from the two genotypes. The absence of percolation in the case of genotype PB235 suggests that fewer reactive end groups were present on the shell for this rubber.

Influence of slow structuring on the mesostructure of NR samples

It has been known for a long time that NR samples stored at room temperature under ambient conditions evolve, becoming harder. Consequently, the P_0

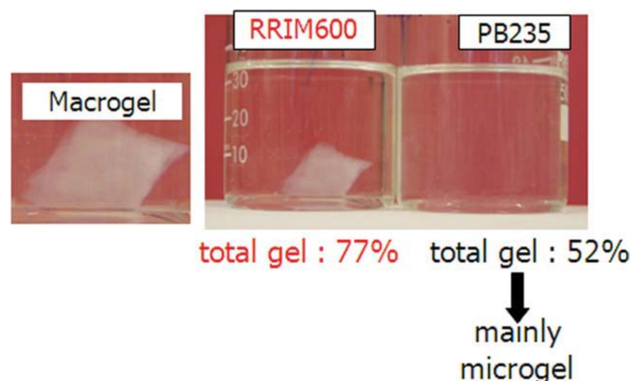

Figure 4 Pictures of flasks after 7 days of solubilization of NR samples from genotype PB235 and RRIM600. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

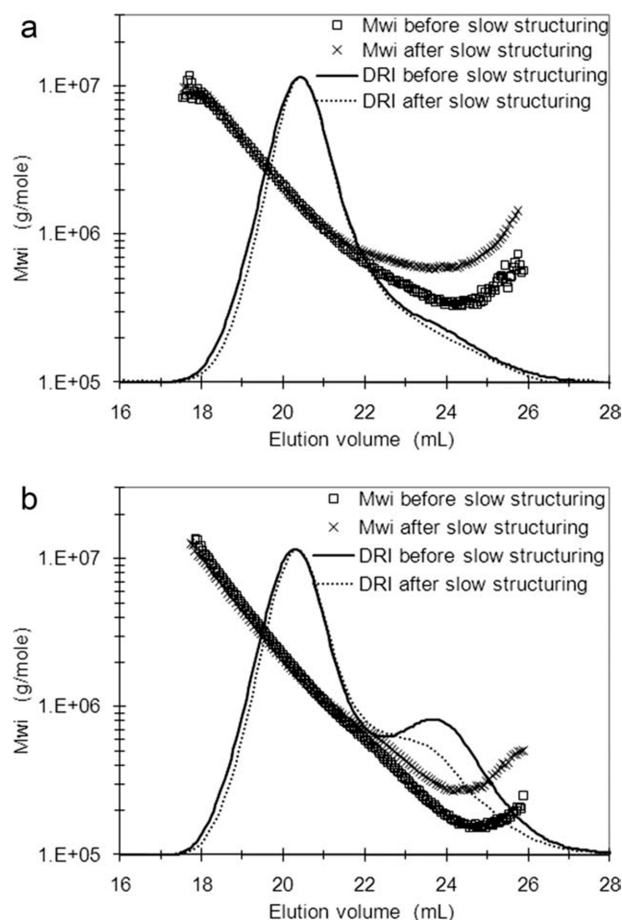

Figure 5 Chromatograms showing the refractometer signal and molar mass (M_{wi}) as a function of elution volume for fresh NR samples stored for 18 months at room temperature (slow structuring) (a) genotype PB235 and (b) genotype RRIM600

and macrogel rate, determined after centrifugation, increases but it has never been investigated how the macromolecular structure evolves. Varghese et al.[8] and Yunyongwattanakorn et al.[7] showed that the P_0 of NR samples increased during storage to reach a plateau between 14[8] and 24 months[7]. We therefore chose a period of 18 months of storage in the laboratory for the slow structuring of our samples. After that period, P_0 increased from 48 to 55 and 36 to 45 for samples from genotype PB235 and RRIM600, respectively, (Table I). The gel rate also increased during slow structuring. The increase of gel rate was about 8% (24–32%) for samples from genotype PB235 and, 8.5% (24.5–33%) for samples from genotype RRIM600 (Table I). For genotype PB235, after slow structuring, as seen in Figure 5A, there was greater M_{wi} abnormality in the zone of the chromatogram corresponding to high elution volumes (22 to 25 mL). As the DRI signal presented no significant change, this phenomenon might again indicate more numerous microaggregates generated in the samples after slow structuring. As a consequence, the M_n significantly increased by about 20%, from 990 kg/mol to 1,176 kg/mol. The samples from genotype RRIM600 exhibited a greater increase in M_n, about 60%, from 530 kg/mol to 870 kg/mol. But again, as the DRI signal show disappearance of short chains, the increase of M_n is mainly due to this phenomenon.

Influence of fast structuring on the mesostructure of NR samples stored for 18 months at room temperature (slow structuring)

Like the fresh samples, these samples stored for 18 months at room temperature underwent fast structuring on P_2O_5. As for the fresh samples, P_0 increased dramatically (Table I) and the values reached displayed no significant difference from those obtained with "fast structured" fresh samples (Table I). The gel rates also increased after fast structuring on P_2O_5, but the values reached were significantly lower than those observed for fresh samples after the same fast structuring (Table I). The stored samples from genotype PB235 had a gel rate of 43% as opposed to 52% for fresh samples after fast structuring (Table I). The difference was greater for samples from genotype RRIM 600, 64% as opposed to 77%. Surprisingly, fast structuring did not lead to the same gel rate results depending on the storage history of the NR samples at room temperature. It seems that the structuring undergone by NR samples at room temperature influenced their consecutive fast structuring on P_2O_5. This point calls for additional experiments to explain it. On the other hand, the storage history at room temperature for the samples from genotype PB235 had no effect on the macromolecular structure after fast structuring on P_2O_5 [Fig. 6(A)]. The average molar masses were the same, as presented in Table I. For the samples from genotype RRIM600, the history of the samples at room temperature had a significant influence on both gel rate, as seen above, and macromolecular structure [Fig. 6(B)].

CONCLUSIONS

Our results showed that fast structuring of NR samples on P_2O_5 caused substantial changes in mesostructure for both genotypes studied. The most significant changes observed were a dramatically increased of M_n and total gel rate after fast structuring. However, the increase in M_n could not be explained in the same manner for both genotypes. In the case of genotype RRIM600, the increase in M_n was explained mainly by a decrease in the number of short chains. For genotype PB235, the increase in M_n was explained mainly by an increase in the quantity or size of microaggregates. In addition,

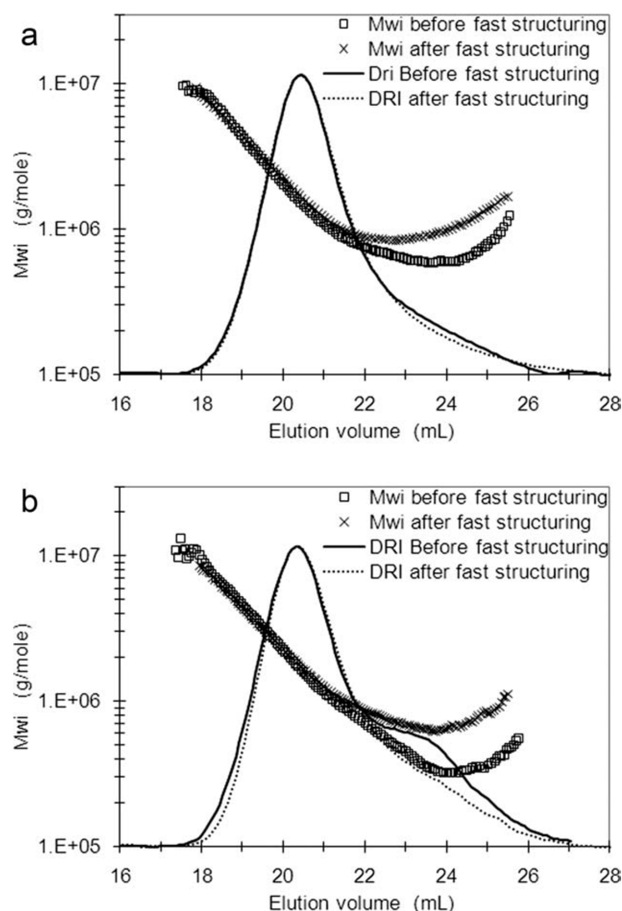

Figure 6 Chromatograms showing the refractometer signal (DRI) and molar masses (M_{wi}) as a function of elution volume for NR samples stored for 18 months (slow structuring) before and after fast structuring on P_2O_5 (a) genotype PB235 and (b) genotype RRIM600

from a qualitative point of view, the gel was very different after fast structuring for both genotypes. Indeed, though samples from genotype PB235 contained 52% of total gel, there was no gel visible in the flask, unlike for the samples from genotype RRIM600 which exhibited a very visible macrogel. The occurrence of percolation between elementary bricks, probably microaggregates, especially under extreme conditions (fast structuring) depends on the genotypes.

Our work also highlighted that slow structuring (18 months' storage at room temperature) of NR samples also led to an increase in M_n and total gel rate, though less drastic compared with fast structuring. In addition, the response of NR samples to fast structuring was different depending on their storage time at room temperature, i.e., having undergone 18 months slow structuring or not. The NR samples left for 18 months at room temperature (slow structuring) exhibited a lower increase in total gel rate after fast structuring than fresh samples.

The authors thank Union Rubber Co. Ltd for providing facilities in their plantation. This work was undertaken as part of the *Hevea* Research Platform in Partnership (HRPP), Thailand.

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