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# Rubber Particles: Size, Molecular Weight and Their Distributions Detected in Wild Hevea Species

Ong Chin Wei[1]* A.R. Shamsul Bahri[2]
1.Crop Improvement and Protection Unit, The Rubber Research Institute of Malaysia
2.Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu, Malaysia

**Abstract**
Rubber cultivation in Malaysia has been extended to large areas all over the country in many decades ago in order to produce high quantity of latex for the rubber industry. The wild *Hevea* species planted and conserved in Rubber Research Institute of Malaysia (RRIM) are collected from South America especially Amazon basin. These wild *Hevea* species are considered as the most valuable genetic materials by many rubber plant breeders for achieving further genetic advances in *Hevea* improvement programmes. Currently, 8 wild *Hevea* species are being conserved in the germplasm conservation area namely *H.benthamiana, H.brasiliensis, H.camargoana, H.guianensis, H.nitida, H.pauciflora, H.rigidifolia* and *H.spruceana*. Generally, studies have been carried out on these wild *Hevea* species focusing on the dry rubber content, mechanical stability, Wallace plasticity and Mooney viscosity. There are no thoughtful investigations into the rubber particle size, molecular weight and their distributions in these wild *Hevea* species for utilization in the crop improvement programmes yet. Therefore, this paper attempts to uncover the characteristics of rubber particles in wild *Hevea* species that never have been explored as potential species in the context of rubber particle size, rubber molecular weight as well as rubber particle size distribution and rubber molecular weight distribution.
**Keywords**: Rubber particles, Size distribution, Molecular weight, Molecular weight distribution

## 1.0 Introduction

The genus *Hevea* belongs to the family Euphorbiaceae but only *H.brasiliensis* is a major source of natural rubber planting material and widely planted in rubber producing countries (Webster and Paardekooper, 1989; Schultes, 1990; Priyadashan, 2011). Most of the wild *Hevea* species grow in the wild forest basically in the Amazon basin, South America. The wild *Hevea* species are distributed and originated from South American countries such as Brazil, Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname and Venezuela (Webster and Paardekooper, 1989). The wild *Hevea* species produce rubber particles that are bounded by membranes of lipids, proteins and other substances that distinctive to other latex-bearing plants (Cornish *et.al*., 1993). In general , many studies of latex characteristics have been focused on the dry rubber content, mechanical stability, Wallace plasticity, Mooney viscosity in *H.brasiliensis*. Nevertheless, the rubber particle size, molecular weight and their distribution in wild *Hevea* species such as *H.benthamiana, H.camargoana, H.guianensis, H.nitida, H.pauciflora, H.rigidifolia* and *H.spruceana* only getting minuscule attention.

Most of the rubber particles appeared as dim dark spheres suspended in the cytosol of laticifer cells that would appear distinctly whenever soaked with chemicals such as osmium tetroxide and staining metals *i.e.* uranyl acetate and lead citrate (Shamsul Bahri *et.al*., 2013). However, rubber particles sometimes appeared as spherical shape in young and immature trees but most rubber particles are often larger and appeared like pear-shaped in mature tree (Gomez and Samsidar, 1980; Shamsul Bahri, 2000). The average size of the rubber particles are different depend on the rubber genotypes or planting materials but at least two distinct subsets of particles with average size of diameters between 1.0 nm and 0.2 nm were found in *H.brasiliensis* (Cornish *et al.*, 1993; Wood and Cornish, 2000). Tangpakdee *et.al.* (1996) observed that structure of rubber particles obtained from young *H.brasiliensis* trees was found to be fundamentally similar as appeared in the mature trees. However, there were different peaks in molecular weight distribution observed in the rubber samples. Subramaniam (1980) reported that three types of polydispersity molecular weight distribution ($M_w/M_n$) were distinguished and rather broad range between 2.5-10 while Eng *et.al*. (2001) detected a narrower range between 3.1-5.4. The observations suggested that different molecular weight distribution might cause by the decrease activities of terminal groups in rubber particle and diverse enzyme activities such as transferase and isomerase. Therefore, rubber particles produced in wild *Hevea* species is believed to be synthesized and regulated by the same activities of rubber particle-associated proteins and enzymes activities found in *H.brasiliensis*. There were several types of molecular weight distribution of rubber found in cultivated *H.brasiliensis*, *i.e.*: (1) bimodal distribution with peaks almost the same height; (2) bimodal distribution with lower peak appeared in the lesser molecular weight region; (3) skewed unimodal distribution with a constant in the lesser molecular weight region.

## 2.0 Materials and Methods

### 2.1 Rubber Particle Size and Rubber Particle Size Distribution

Latex samples from eight different *Hevea* species *viz*. *H.brasiliensis*, *H.benthamiana, H.camargoana,*

 

*H.guianensis, H.nitida, H.pauciflora, H.rigidifolia* and *H.spruceana* were collected in the early morning before 7 a.m. and transported to laboratory. The latex samples of each wild *Hevea* species was contained in an Eppendorf tube and treated with 1% osmium tetroxide by 1 droplet (about 1 ml). The samples were left undisturbed for about 1 hour so that complete fixation between osmium tetroxide and latex could be accomplished. Later, these latex samples were centrifuged at 10,000 rpm for 5 minutes and the supernatant after the centrifuge were discarded. Following that, 1 ml of distilled water was pipetted into each sample to eradicate excess osmium tetroxide that might attached in the latex. Then, the samples were ready for another centrifuged at 10,000 rpm for 5 minutes. Afterwards, a wire loop was used by dipping it into the centrifuged suspension and a thin layer of latex suspensions would be formed on the wire loop inside the Eppendorf tube. This step was repeated for every wild *Hevea* species latex sample. The thin layer of suspensions were then relocated from their Eppendorf tubes onto the colloid coated-copper grids and were left to dry at room temperature. In the end , the grids with thin layer of suspension were observed under the Philips CM12 Transmission Electron Microscope (TEM) at 80 kV with at least 15,000 magnification. The morphological properties and size distribution of rubber particles from eight different wild *Hevea* species were then examined using an image analyzer SiS Imagine software that attached to the transmission electron microscope.

### 2.2 Rubber Molecular Weight and Rubber Molecular Weight Distribution

Fresh latex from eight different wild *Hevea* species were collected in the early morning not latter than 8 a.m. in the field and transferred to laboratory. All samples with equivalent of 2-3 ml were smeared as a thin layer on a clean glass surface. The area smeared would covered an area about 6 x 6 inches and left to dry at room temperature before being scraped off from the glass surface. At this stage, the dried thin rubber films were formed from the latex samples and then were dissolved into Liquid of Tetrahydrofuran (THF) at 0.1-0.15 % w/v. These dissolved samples were left for about 24 hours in the dark room. Later, these samples were filtered through a 0.45 µm filter and measured by Gel Permeation Chromatography (GPC). In the GPC measurement, a system consisting of Water 2690 Separation Module and Water 410 differential refractometer were applied to the filtered samples. These samples would be separated through three stages of styrene-divenyl benzene gels packed columns that connected in sequence with the exclusion limit of $4x10^5$, $4x10^6$ and $2x10^8$ respectively. Commercial polyisoprene was used as the standard to calibrate the columns. Measurements were made at temperature about 40˚ C with approximately 0.8ml/min flow rate in the mobile phase. Eventually, the rubber particles would be separated by size and their molecular weights were estimated as polystyrene equivalent values. Biochemical characteristics such as number-average molecular weight, weight-average molecular weight, maximum peak of molecular weight, polydispersity and type of molecular weight distribution in wild *Hevea* species could be determined by GPC method.

## 3.0 Results and Discussion

### 3.1 Rubber Particle Size and Rubber Particle Size Distribution

Figure 1 showed the transmission electron micrographs of microstructure of rubber particles used for determination of rubber particles size and their distribution. The results obtained from the samples showed that most of the wild *Hevea* species presented their rubber particles as the spherical shape, regardless their particle size, either they were large or small rubber particles. Nevertheless, *H.benthamiana* and *H.brasiliensis* revealed that their rubber particles were appeared as large rubber particles with prominent pear-shape (elongated) besides the ordinary spherical (oval) shape rubber particles. Besides *H.benthamiana* and *H. brasiliensis*, the large rubber particles seemed to be scarce or unlikely to be detected in other wild *Hevea* species. There was no agglomeration (heavily clustered) of rubber particles or in all samples observed except a slightly clustered of rubber particles detected in *H. rigidifolia*. As the latex samples is this study were collected from the field and not the ready-made centrifuged latex that could separated into several zones according to their weight and size, therefore the rubber particles with both small and large were seen to be intermingled with each other with no distinguishing proportion.

Figure 2 - 3 showed that the mean diameters of rubber particles in eight *Hevea* species. The results showed that the diameter of rubber particles varied in these eight wild *Hevea* species were ranged in between 350 nm and 700 nm (0.35 µm - 0.7 µm). Rubber particles size in *H. benthamiana, H.brasiliensis, H camargoana, H nitida* and *H.guianensis* were in the range in between 500 and 700 nm (0.5 - 0.7 µm) though *H.pauciflora, H.rigidifolia* and *H.spruceana* were below 400 nm (0.4 µm) in particle size. The difference in the range of particle size is believed to contribute in the ability of wild *Hevea* species to develop their latex for final rubber products where the size of rubber particles might influence the molecular weight of their rubber particles in the latex. Rubber particles with different particle shape, size and particle size distribution might contribute to the unique signature of the wild *Hevea* species and most probably their end products too. Not like other wild *Hevea* species, rubber particles in *H.brasiliensis* and *H.benthamiana* showed abundance of pear shape rubber particles along with other oval shape rubber particles. This prominent characteristic most probably indicated a different

 

mode of origin for latex biosynthesis in *H.brasiliensis* and *H.benthamiana* that was not found in other wild *Hevea* species.

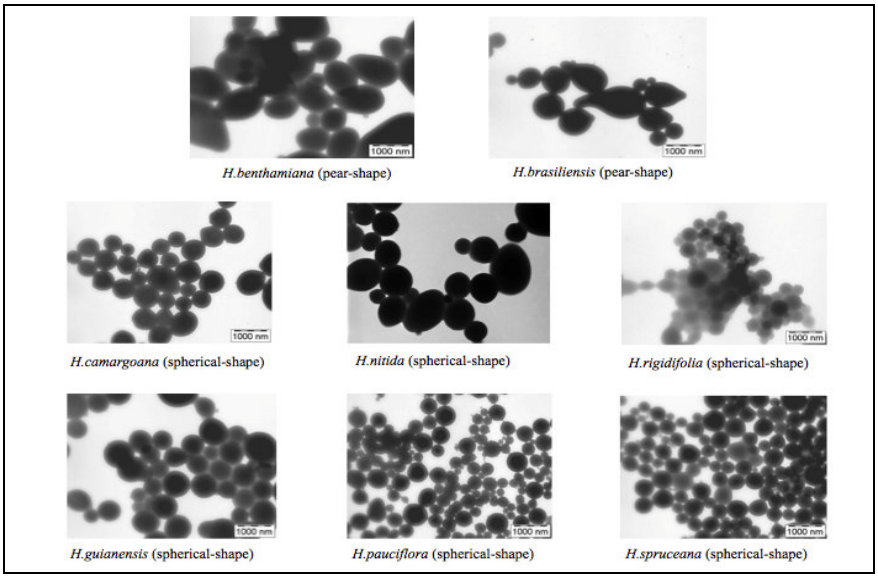


Figure 1. The transmission electron micrographs of rubber particles in wild *Hevea* species.

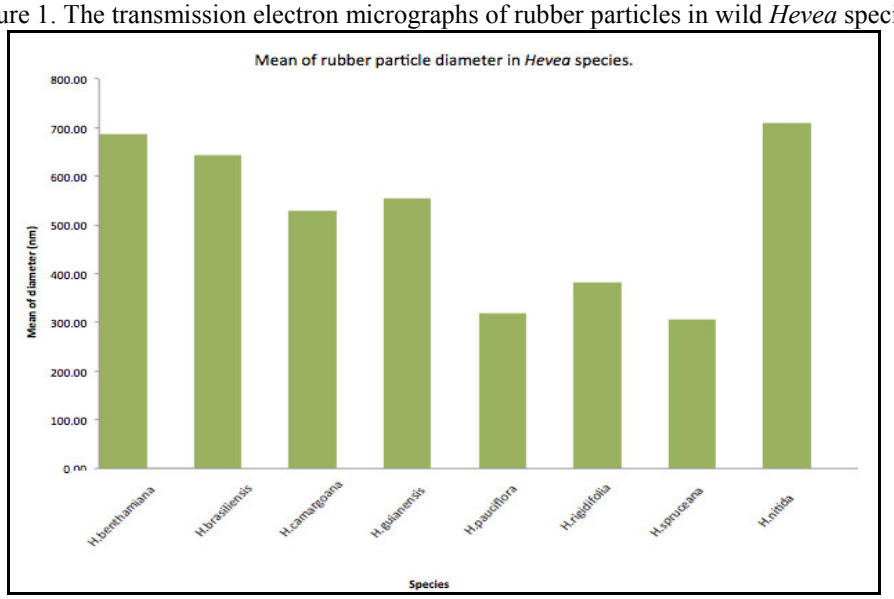


Figure 2. Mean of rubber particle diameter in *Hevea* species.

 

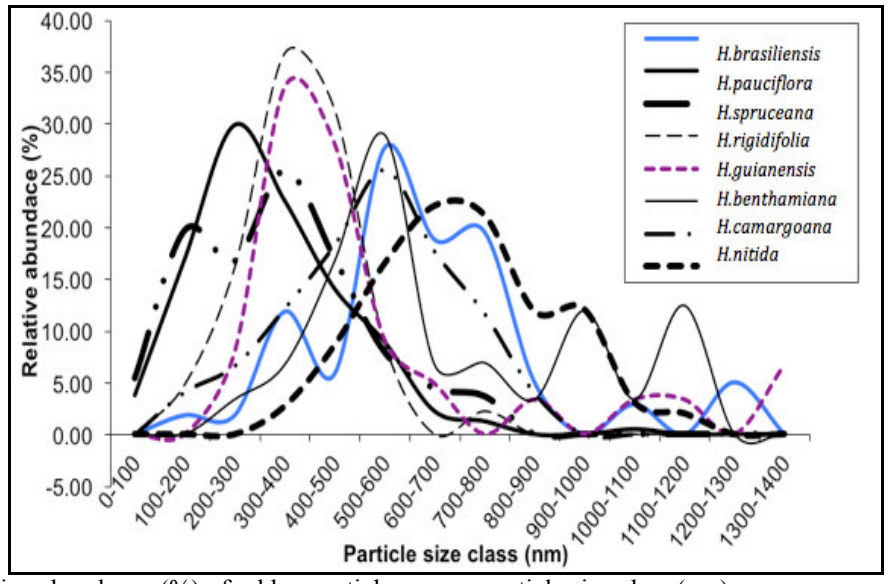

Figure 3. Relative abundance (%) of rubber particles versus particle size class (nm).

### 3.2 Rubber Molecular Weight and Rubber Molecular Weight Distribution

The molecular weight distribution of natural rubber is very wide and this is true for all wild *Hevea* species observed in this study (Table 1). This observation is similar to the molecular weight distribution (MWD) in several selected clones of *Hevea brasiliensis* as reported earlier by Subramaniam (1980). The analysis of these wild *Hevea* species revealed that there was a range of molecular weights (Mw) exhibited from 1.52 x $10^5$ (*H.camargoana*) to 16.35 x $10^5$ (*H.brasiliensis*) among t hemselves. *H.brasiliensis* showed the highest value in both number and weight in the mean of molecular weight in gel permeation chromatography. The polydispersity (Mw/Mn) value of rubber particle showed a diverse values from the highest at 6.02 (*H.brasiliensis*) to the lowest at 2.92 (*H.nitida*). This study shows that natural rubber for all the eight *Hevea* species may in general be considered to have a bimodal distribution. It was found that the molecular weight distribution of wild *Hevea* species could be documented into one of the three classes polydispersity as shown in Table 1. The type of polydispersity is this study is constructed on the research that have been carried out by Subramaniam (1980), Hager *et.al*. (1979), Tangpakdee *et.al*. (1996) and Eng *et.al*. (2001).

Basically, there were three classes of polydispersity distribution observed in this study where an obvious bimodal distribution with peaks very near to each other or often almost the same height to each other that catalogued as Type 1. On the other hand, an obvious bimodal with one peak distributed at the lesser molecular weight region while another peak distributed in the higher molecular weight region that catalogued as Type 2. The last type was a skewed unimodal distribution with a constant or almost constant in the lesser molecular weight region and catalogued as Type 3. There were slightly different in the molecular weight distribution observed in wild *Hevea* species where most of the species exhibited Type 2 molecular weight distribution in the exception of *H.camargoana* (Type 1) and *H.nitida* (Type 3). *H.brasiliensis* and *H.benthamiana* show a type 2 MWD curves with the bimodal distribution where peak in the low molecular weight region usually higher than the other one. The bimodal distribution is most probably to be the sum of two different unimodal distributions that arising from two distinctive enzyme systems in the rubber particle biosynthesis where each of the system contributed to the two region of molecular distribution. Out of the eight wild *Hevea* species in this study, only *H.nitida* exhibits type 3 MWD curve with unimodal distribution with almost constant value skewed in low molecular weight region. The tendency of higher proportion of higher molecular weight rubber particle in the unimodal indicated that high possibility of the particular enzyme system that has excessive influenced in the biosynthesis of high molecular weight rubber in *H.nitida*.

 

Table 1. Mean of molecular weight (Mw) and type of molecular weight distribution (MWD) curve from the fresh latex of wild *Hevea* species.

| No | *Hevea* species | $M_n$ (x $10^5$) From GPC | $M_w$ (x $10^5$) From GPC | MP (x $10^5$) | Polydispersity ($M_w/M_n$) | Type of MWD curve |
|---|---|---|---|---|---|---|
| 1. | *H.benthamiana* | 2.56 | 14.03 | 14.75 | 5.49 | 2 |
| 2. | *H.brasiliensis* | 2.72 | 16.35 | 26.36 | 6.02 | 2 |
| 3. | *H.camargoana* | 1.71 | 15.20 | 1.38 | 8.92 | 1 |
| 4. | *H.guianensis* | 0.96 | 4.25 | 1.14 | 4.42 | 2 |
| 5. | *H.nitida* | 0.94 | 2.73 | 1.23 | 2.92 | 3 |
| 6. | *H.pauciflora* | 0.96 | 6.35 | 1.16 | 6.63 | 2 |
| 7. | *H.rigidifolia* | 1.43 | 7.02 | 1.72 | 4.92 | 2 |
| 8. | *H.spruceana* | 1.24 | 7.60 | 1.44 | 6.14 | 2 |

Note: $M_n$: number-mean molecular weight, $M_w$: weight-mean molecular weight, MP: maximum peak MWD curve:
Type 1: Distinctly bimodal distribution, peaks of nearly the same height.
Type 2: Distinctly bimodal distribution, peak in low molecular weight region is smaller.
Type 3: Skewed unimodal distribution with 'plateau' in low molecular weight region.

**4.0 Conclusions**

Primary characteristic of latex is usually found in the content of laticifers. Rubber particles with different particle shape, size and particle size distribution contribute to the unique signature of each *Hevea* species. Unlike other species, rubber particles in *H. brasiliensis* and *H. benthamiana* for example showed large rubber particles with pear-like structure very prominently and probably suggested the mode of origin (latex biosynthesis) for rubber. Rubber particle mean of diameter for all species varies between each other. However, this study demonstrates there is no pre-determination between rubber molecular weight and rubber particle size in wild *Hevea* species but the possible connection between them should not be ruled out due to the unclear probability of the initiation and termination mechanisms of rubber formation. This study has observed at least three classes of molecular weight distribution in wild *Hevea* species as the same classes that showed by commercial planting materials in previous observations. The bimodal distribution would be expected to give natural rubber a satisfactory compromise between properties and process ability. The high molecular weight portion is thought to give the rubber strength and other essential physical properties while the low molecular weight fraction acts as a plasticizer and enable rubber to be processed more efficiently and easier, especially in the early stages of rubber particle breakdown to achieve high rubber blending compatibility (Subramaniam, 1972; Hager *et.al*., 1979; Eng *et.al*., 2001; Loyens and Groeninckx, 2002; Gronover *et.al*., 2011). According to Hager *et.al*. (1979) and Tangpakdee *et.al*. (1996), molecular weight distribution of rubber particle is mostly detected as bimodal distribution in commercial planting materials based on gel permeation chromatography experiments and the results obtained in this study is supporting their findings eventhough the rubber particle samples are collected from wild *Hevea* species. This finding implies that rubber molecular in bimodal distribution are formed as branched chains from both low and high branched rubber molecules. The important characteristics such as high rubber particle size and high rubber particle size distribution can be explored as the indicators for high latex production in the crop improvement programmes while high rubber molecular weight and bimodal molecular weight distribution are preferable for rubber processing in the factory. Besides *H.brasiliensis*, *H.benthamiana* should be included as one of the most potential *Hevea* species for latex production in the future crop improvement programmes.

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**About authors:**

**Ong Chin Wei** obtained his Bachelor of Crop Science in 2002 at University of Putra, Malaysia. He received a Master Science of Crop Improvement (Merit) from University of Nottingham, United Kingdom in 2010. He is a plant breeder and rubber clone inspector at The Rubber Research Institute of Malaysia (RRIM) and experience in rubber clone improvement and rubber genetics study.

**A.R. Shamsul Bahri** obtained his PhD from University of Durham, United Kingdom in the field of Plant cell biology. He has been involved in Microscopy and anatomical studies since 1992 and gained numerous skills in Materials Characterization and Plant Improvement and Protection over the years. Currently, Dr.Shamsul teaches plant breeding, plant biology and physiology as well as microscopy studies at The School of Food Science and Technology in Universiti Malaysia Terengganu.