J. nat. Rubb. Res., 4(3), 204–211

Particle Size Distribution in Hevea *Latex — Some Observations on the Electron Microscopic Method*

J.B. GOMEZ AND SAMSIDAR HAMZAH*

Hevea *latex is a hydrosol in which rubber occurs in the form of discrete particles in a diameter range of 50 Å — 3 μ. The particle size distribution in forty clones was investigated and their various diameters were determined by a standardised electron microscopic method. Number average diameter varies from 942 — 1858 Å and this can be influenced by changes in the exploitation system such as the stimulation practice.*

It is shown that electron microscopy is a reliable method for particle size studies on latices if modern methods are used. A computerised measurement system will no doubt speed up the tedious manual procedures used in this paper.

Hevea latex is a hydrosol in which rubber occurs in the form of discrete particles in a diameter range[1] of 50 Å — 3 μ. The small particles are spherical but the larger ones are often pear-shaped in certain clones.

Previous studies on particle sizes using light microscopy[2], ultra-violet light microscopy[3,4] and electron microscopy[5,6] have increased our knowledge and understanding of particle size distributions in *Hevea* latices. The earlier light microscopic work is not reviewed here, but some of the electron microscopic (E.M.) work has to be reconsidered for a better understanding of the problem.

Schoon and van der Bie[5] studied brominated *Hevea* latices from several clones and arrived at the conclusion that for each latex, particles were distributed in several Gaussian distributions. They deduced that all observed particle sizes were multiples of 580 Å or 690 Å. From this, they were of the opinion that larger particles were grape-like clusters of smaller particles. In a later paper, Schoon and Phoa[6] assumed that the bromination technique swelled the particles by 100% and hence concluded that particle sizes were multiples of 300 Å sub-particles.

This study gives an appraisal of the E.M. techniques used for the examination of particle size distribution in *Hevea* latices.

MATERIALS AND METHODS

Latex from potted seedlings was obtained by pricking them with needles a few centimetres above the soil level and the ensuing drop of latex was mixed with a drop of fixative at the end of a dropper which contained a few drops of fixative. The latex was drawn into the dropper and immediately fixed. After a suitable duration in 1% osmium tetroxide solution for adequate hardening of the particles; the material was transferred to pre-coated E.M. grids using a wire loop method. After drying, the grids were washed in water, face downwards and without much agitation. The grids were dried prior to electron microscopy.

Latices for E.M. examination when obtained from mature trees were collected under chilled conditions from six to eight trees per clone and brought to the laboratory for fixation and preparation of E.M. grids. Latex was collected for 30 min after tapping. The tapping was under the ½S d/2 system. Fixation was for a normal duration of 2 h and after that low-speed centrifugation was used to concentrate the particles and wash the suspensions free from osmic acid before final E.M. examination using a Philips EM100 or Philips EM300.

The effects of stimulation by ethephon were studied on forty clones growing in the nursery. The trees were stimulated with 10% ethephon

*Rubber Research Institute of Malaysia, P.O. Box 10150, Kuala Lumpur, Malaysia

applied to scraped bark below the ½S tapping cut. Latex was collected prior to stimulation, two weeks after stimulation and three months after stimulation.

At least 1000 particles were measured for each clonal latex to obtain a statistically random sample from an infinite population. The measurement was done on E.M. photographs taken at a magnification of 50 000. Several operators contributed to the observations reported here. Operator error was however, not studied thoroughly but an assessment of the reproducibility of results was made using different operators.

RESULTS AND DISCUSSION

Figure 1 shows an electron micrograph of a typical preparation used for the determination of particle sizes. The figure shown is at a magnification of 25 000× but in actual size determinations, enlargements at 50 000× were found to be necessary to increase the accuracy of measurement.

Figure 2 shows the particle size distribution in potted seedlings of one-to-two months old. They were examined both before and after stimulation with 2-chloroethyl phosphonic acid (ethephon). In both cases, a bimodal distribution of particle size was observed with the first mode around 800 Å and the second mode around 2400 Å.

Figure 3 shows a summary of observations made on forty clones in the nursery which were stimulated with ethephon at an early age (three years). The determinations of particle sizes were made before treatment, two weeks after ethephon treatment and three months after ethephon treatment. The particle size distribution was unimodal in all cases but the peak values of the modes were different in each of

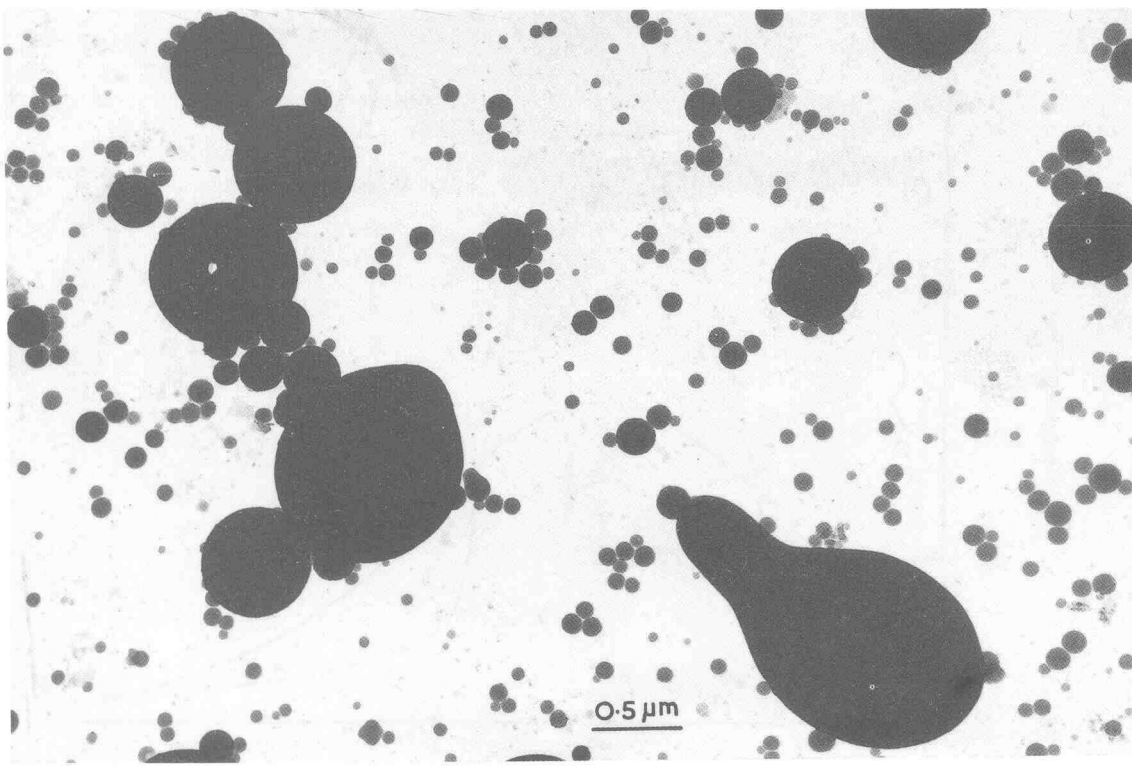

Figure 1. Electron micrograph of a whole-amount latex preparation. Magnification 25 000 ×.

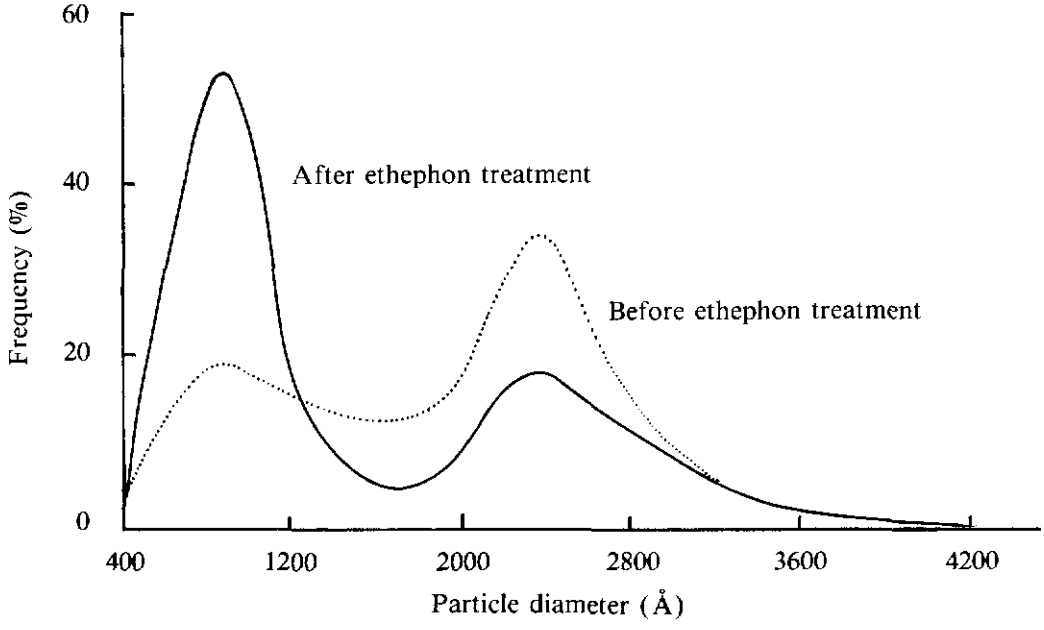

Figure 2. Latex particle size distribution in potted seedling plants.

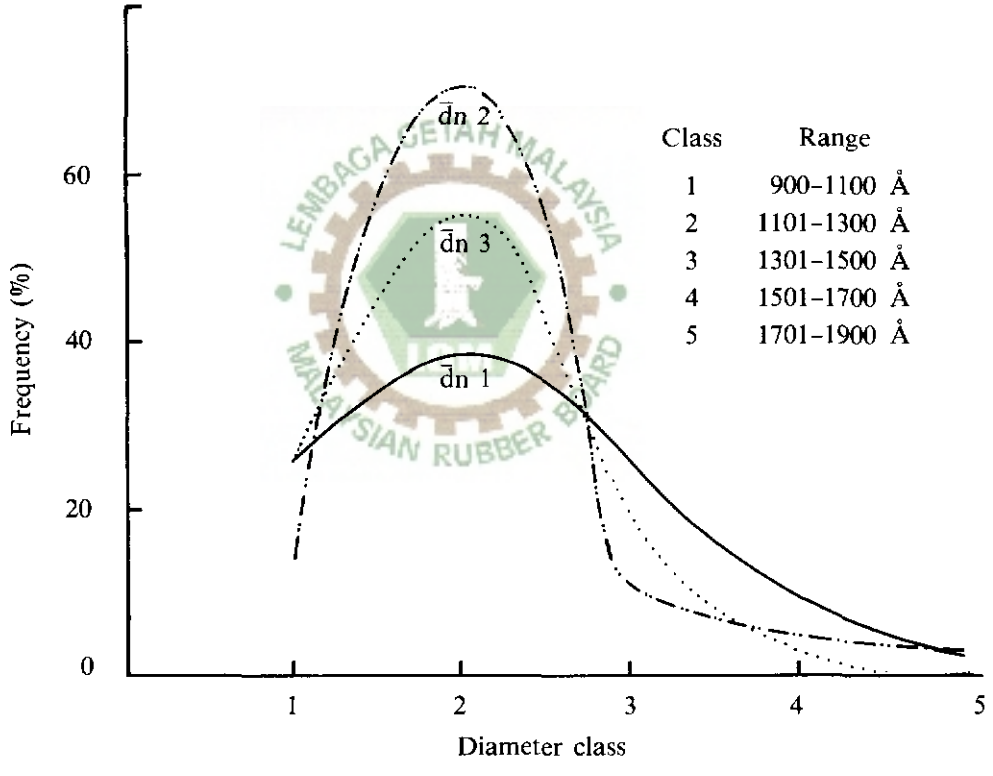

Figure 3. Latex particle size distirbution in forty clones growing in the nursery.

the three instances measured. Before ethephon treatment, the peak frequency at the mode was 38%; two weeks after treatment it was 70% and three months after treatment, it was reduced to 55% but did not go back to the resting mode of 38% as observed before ethephon treatment.

Figures 4–7 show the detailed values for four selected clones from the forty studied. For clone GT 1 *(Figure 4),* the peak values at the mode were only marginally different, again showing the similarity in behaviour to the group of clones studied; *i.e.* before treatment, the peak frequency was about 61%; two weeks after treatment, it was 71% and three months after treatment 66%. RRIM 623 did not behave in the same manner, there was continuous increase in the peak frequency for the three intervals studied. In ES 8, the reverse trend was observed *i.e.* peak frequency was greater before treatment and decreased by about 10% two weeks after treatment and recovered by 6% after three months. RRIM 636 showed a value of 65% before treatment which decreased to 56% two weeks after treatment and recovered to a value of 65% three months after treatment.

Table 1 shows the number average diameter, surface average diameter and volume average diameter for the forty clones kept under observation for each of the three periods of study.

It is clear that electron microscopy offers a good method for estimating particle sizes of *Hevea* latices. The number average diameter varies from a low of 942 Å to a high of 1858 Å in the forty clones studied. The percentage of particles above 4000 Å was less than 7.0% in one clone and even as low as 1% in another clone. Thus, the majority of particles are very small. The principal peak varies between 71% and 40% in various clones.

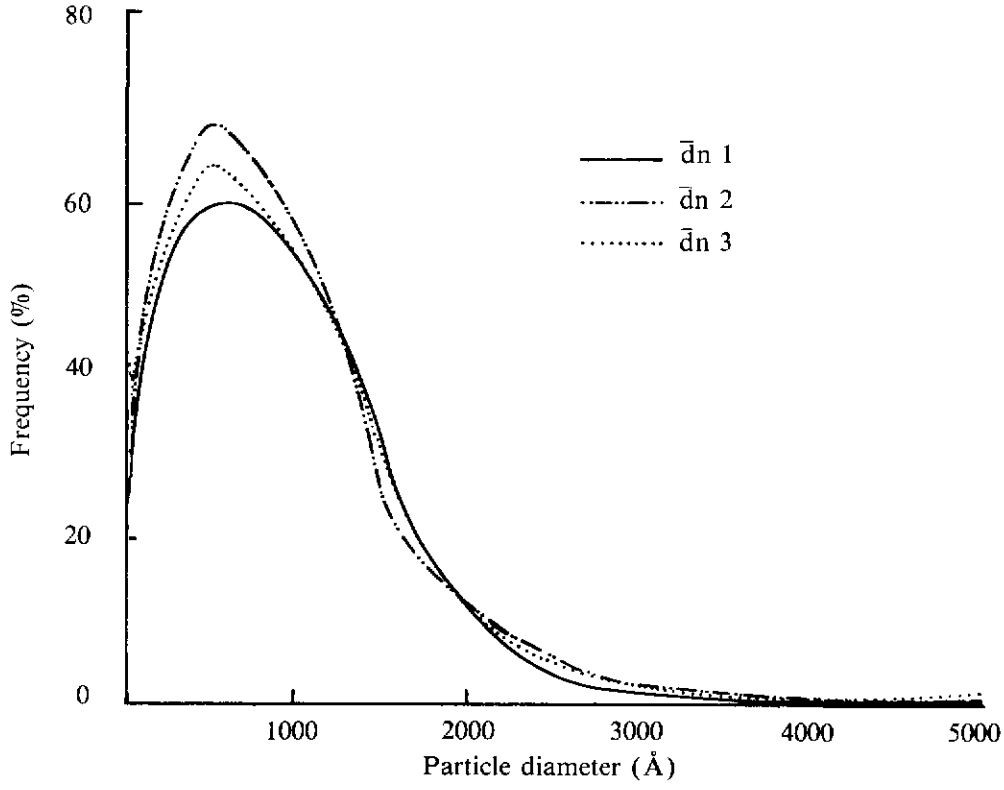

Figure 4. Latex particle size distribution in clone GT 1.

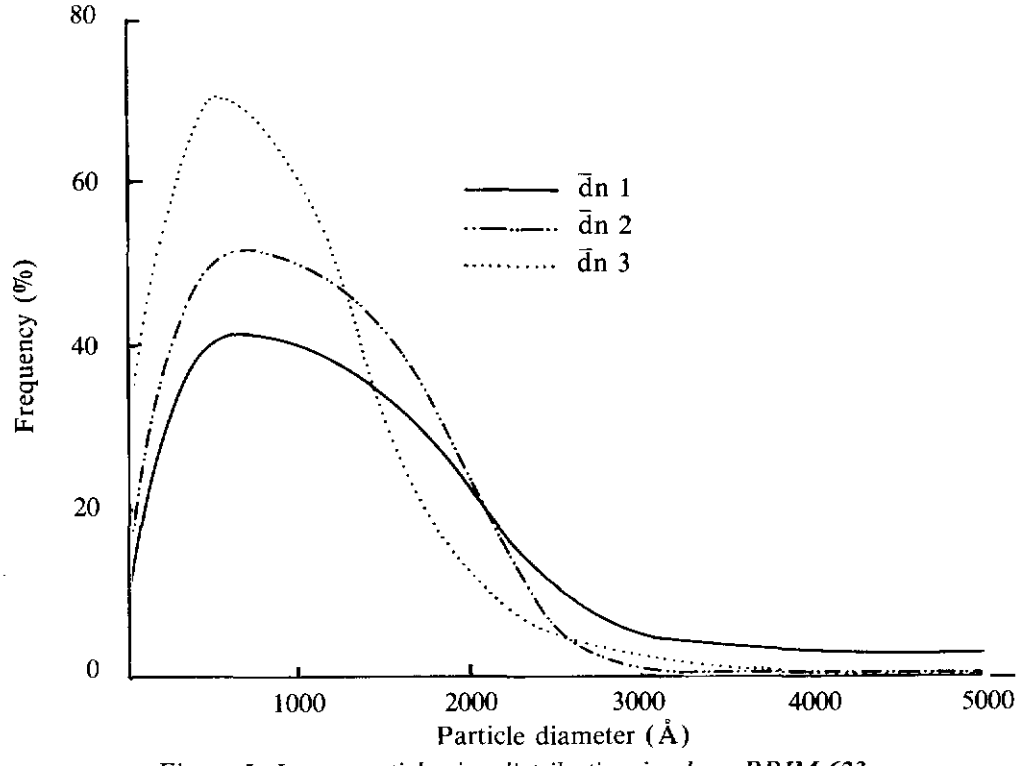

Figure 5. Latex particle size distribution in clone RRIM 623.

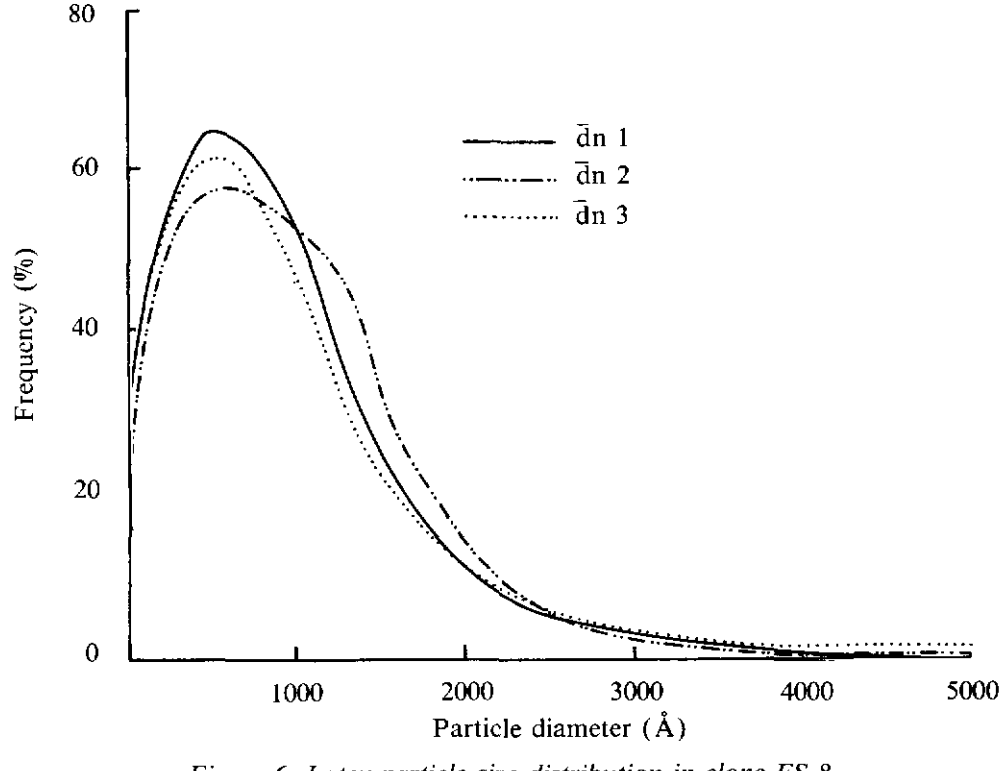

Figure 6. Latex particle size distribution in clone ES 8.

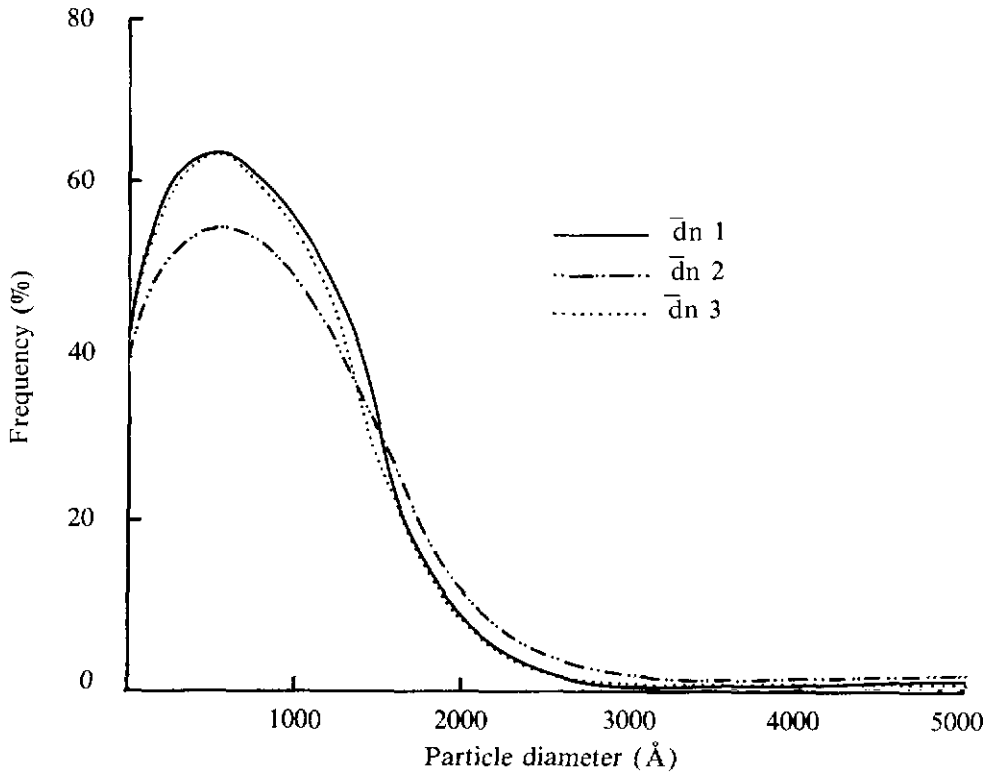

Figure 7. Latex particle size distribution in clone RRIM 636.

The hetero-dispersity of the rubber particle population in *Hevea* latices is well known from past studies. What is interesting is that in most cases observed except in the case of a seedling, the distribution was unimodal with the principal peak occurring between 1100 Å and 1300 Å with a long tail following in the higher diameter classes. In this study however, the clonal differences in particle size distribution were prominent. Apart from a considerable variation in the mean particle size, the clonal variation was also reflected in the position of the mode in the frequency distribution pattern. Yield stimulation with ethephon influences this pattern as well in a way to reflect the increase in the number of smaller particles in the population. Taking into account the diameter classes, *Classes 1, 2* and *3* were predominantly represented prior to the application of stimulant whereas two weeks after treatment, *Class 2* was predominant over the others, showing a value of 70%. Three months after treatment, *Class 2* still predominated, but to a lesser extent than in the period two weeks after stimulation. These results therefore indicate that one of the changes in the tree due to yield stimulation is the formation or mobilisation of smaller particles in the output of latex. It is also possible to infer that the influx of smaller particles is an event which can be detected in the first sampling two weeks after stimulant application during the time of positive yield response in the nursery trees. There is reason to believe that this change is transitory as in the next sampling made three months after stimulation, the proportion of *Class 2* had decreased to a level indicating a pattern of recovery to the original unstimulated state.

In the electron microscopic method used for particle size determinations, only the rubber hydrocarbon particles are visualised. In other methods like that of soap titration, contamination from lutoid particles and Frey Wyssling

TABLE 1. LATEX RUBBER PARTICLE CHARACTERISTICS

Sample	Clone	No. average diameter $\bar{X}$ (Å)			Surface average diameter $\bar{X}s$ (Å)			Volume average diameter $\bar{X}v$ (Å)		
		$\bar{d}n$ 1	$\bar{d}n$ 2	$\bar{d}n$ 3	$\bar{d}n$ 1	$\bar{d}n$ 2	$\bar{d}n$ 3	$\bar{d}n$ 1	$\bar{d}n$ 2	$\bar{d}n$ 3
1	RRIM 501	1 346	1 028	1 038	1 698	1 494	1 520	2 176	2 146	2 260
2	RRIM 513	1 108	1 292	1 158	1 476	1 692	1 458	2 084	2 328	1 988
3	RRIM 519	1 192	1 116	1 210	1 554	1 446	1 726	2 106	2 018	2 456
4	RRIM 600	1 084	1 320	1 200	1 380	1 744	1 714	1 950	2 324	2 456
5	RRIM 605	1 272	1 220	1 212	1 802	1 662	1 840	2 526	1 708	2 692
6	RRIM 612	1 024	1 418	1 254	1 330	1 844	1 746	1 804	2 460	2 496
7	RRIM 614	1 178	1 230	1 162	1 562	1 648	1 552	2 144	2 312	2 476
8	RRIM 615	1 074	1 206	1 066	1 486	1 524	1 490	2 140	2 076	2 354
9	RRIM 623	1 858	1 216	1 138	2 574	1 524	1 586	3 336	2 046	2 274
10	RRIM 632	1 106	1 186	1 234	1 500	1 530	1 758	2 148	2 118	2 452
11	RRIM 636	1 038	1 230	1 076	1 316	1 714	1 534	1 782	2 590	2 192
12	RRIM 638	1 368	1 066	1 138	1 744	1 496	1 500	2 294	2 182	2 056
13	RRIM 701	1 244	1 238	1 194	1 554	1 722	1 834	2 020	2 446	2 710
14	RRIM 707	1 104	1 204	1 218	1 398	1 548	1 602	1 868	2 074	2 190
15	PR 107	1 598	1 232	1 506	2 122	1 758	2 096	2 802	2 636	2 756
16	PR 226	1 356	1 114	1 196	1 966	1 626	1 812	2 740	2 366	2 578
17	PR 231	1 466	1 220	1 494	1 948	1 736	2 256	2 566	2 390	3 082
18	PR 251	1 190	1 178	1 234	1 758	1 564	1 912	2 552	2 104	2 880
19	PR 252	1 292	1 126	1 048	1 748	1 536	1 516	2 406	2 242	2 250
20	PR 255	1 578	1 174	1 248	2 008	1 414	1 756	2 556	1 764	2 600
21	PR 259	1 262	1 218	1 262	1 694	1 676	1 694	2 338	2 042	2 458
22	PB 5/51	942	1 238	984	1 248	1 636	1 422	1 824	2 190	2 202
23	PB 5/63	1 430	1 234	1 076	2 036	1 622	1 538	2 822	2 196	2 246
24	PB 28/59	1 302	1 730	1 308	1 800	2 364	1 922	2 482	3 540	2 830
25	PB 86	1 150	1 100	1 308	1 464	1 424	1 672	1 912	2 062	2 196
26	GT 1	1 094	986	1 214	1 376	1 338	1 806	1 854	1 660	2 604
27	AVROS 385	1 302	1 552	1 336	1 826	2 074	1 872	2 490	2 752	2 664
28	AVROS 427	1 076	1 094	966	1 366	1 516	1 422	1 842	2 142	2 078
29	AVROS 1279	1 144	1 240	1 170	1 476	1 750	1 526	2 008	2 492	2 106
30	AVROS 1350	1 186	1 200	1 164	1 638	1 614	1 586	2 392	2 280	2 238
31	AVROS 2037	1 010	1 134	1 054	1 394	1 506	1 408	2 154	2 216	2 078
32	RRIC 6	1 120	1 142	1 044	1 432	1 570	1 436	1 924	2 200	2 050
33	RRIC 14	980	1 318	1 188	1 370	1 760	1 704	2 008	2 378	2 620
34	RRIC 36	942	1 162	1 100	1 304	1 536	1 500	1 984	2 052	2 160
35	LCB 1320	1 486	1 500	1 120	2 212	2 016	1 500	3 086	2 708	2 122
36	TR 1514	1 568	1 192	1 350	2 062	1 670	1 696	2 738	2 256	2 144
37	TR 3702	1 352	1 250	1 132	1 686	1 630	1 450	2 202	2 198	1 952
38	ES 5	1 522	1 110	1 308	2 112	1 582	1 968	3 030	2 302	2 992
39	ES 8	1 230	1 218	1 416	1 832	1 574	2 236	2 678	2 136	3 148
40	IRCI 9	1 384	1 528	1 258	1 798	2 062	1 642	2 348	2 726	2 182

complexes or their sub-units created by the dispersive activities of the soap used in the experiment is likely to interfere in the particle size measurement, unless ammoniated latices are used where these particles would have been disrupted into smaller units. Even in other techniques of particle size analysis, there is no direct visualisation of the constituent units. Consequently, it is proposed that the electron microscopic method should be used as the standard for comparison of other methods.

CONCLUSIONS

In previous studies, it was suspected that bromination introduces an artefact in the form of swelling of particles. The use of osmium tetroxide does not present these problems and the particles are sufficiently hardened by the method to withstand electron bombardment and consequent effects on the soft polymer particles. Our trials with osmium tetroxide and other electron stains such as bromine, iodine, *etc.* revealed that osmium tetroxide as a vapour or as a solution is most effective in 'fixing' rubber particles and rendering them sufficiently hard for further observation under the hot atmosphere of the specimen chamber of the electron microscope. The stability of the preparations under electron bombardment is also of no concern when osmium tetroxide is used as the 'fixative'.

The statistics reported in this paper should be taken as an indication that the electron microscopic method is a reliable method for particle size studies. The measurement of particle size from electron micrographs was very tedious as computerised methods were not used. This method is definitely impractical for large-scale applications such as those required in routine latex laboratories.

ACKNOWLEDGEMENTS

Several assistants in the Electron Microscopy Group provided technical assistance in the preparation of electron micrographs and the measurement and tabulation of the data. Computational assistance of Encik R. Surendran is deeply appreciated. Puan Sooryakumari's help in the typing of the script is also acknowledged.

Date of receipt: May 1989
Date of acceptance: July 1989

REFERENCES

1. GOMEZ, J.B. AND MOIR, G.F.J. (1979) The Ultracytology of Latex Vessels in *Hevea brasiliensis*. *Malay. Rubb. Res. Dev. Bd. Monogr. No. 4.*
2. LANGELAND, E.E. (1936) A New Rubber Particle Count in *Hevea* Latex. *Ind. Engng Chem. analyt. Edn.*, **8**, 174.
3. LUCAS, F. (1938) Ultraviolet Microscopy of *Hevea* Rubber Latex. *Ind. Engng Chem.*, **30**, 146.
4. VAN DEN TEMPEL, M. (1952) Electron Microscopy of Rubber Globules in *Hevea* Latex. *Trans. Instn Rubb. Ind.*, **28(6)**, 303.
5. SCHOON, TH. G.F. AND VAN DER BIE, G.J. (1955) Particle Size Distribution in Brominated *Hevea* Latices. *Arch.v.d. Rubbercult.*, **32**, 329.
6. SCHOON, TH. G.F. AND PHOA, K.L. (1956) Morphology of Rubber Particles in Natural Rubber Latices. *Arch. v.d. Rubbercult.*, **33**, 195.