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Distribution of Crosslinks between the Phases of Vulcanised Natural Rubber/ cis-Poly(butadiene) Blends

S.A. GROVES*# AND A.J. TINKER*

A swollen-state NMR method of rubber blend analysis has been used to study crosslinking in gum vulcanisates for cure times ranging from t_{30} to overcure. Natural rubber (NR), cis-*poly(butadiene) (BR) and NR/BR blend vulcanisates cured with conventional and semi-EV sulphur systems based on three common sulphenamide accelerators were studied. For single polymers, maximum crosslink densities were similar for a given elastomer regardless of accelerator. The crosslink densities attained in the blend phases at maximum crosslinking were close to those expected from single polymer data, but the manner in which this was achieved was rather different from expected from cure behaviour of the single polymers. NR has a shorter scorch time than BR, but results indicate that in blend materials the BR phase begins curing before and at a much faster initial rate than NR. The cure rates are reversed as the cure progresses and the NR phase eventually attains the crosslinking levels expected from single polymer data.*

INTRODUCTION

Crosslink formation in the separate phases of a polymer blend is an important factor contributing to the final properties of the vulcanised material. The solubilities, reactivities, and diffusion rates of the crosslinking agents greatly influence the crosslink levels within each phase. The development of a swollen-state NMR spectroscopy technique[1–4] has allowed the estimation of crosslink densities in the individual phases of vulcanised blends to be determined. The technique is based upon the observation that NMR peak line widths increase smoothly with increasing physical crosslink density (n_{phys}) or other parameters related to this such as curative level or volume fraction of rubber in the equilibrium swollen state (v_r). Line width is measured as the proportion of signal strength at a reference position to that at the peak, and is denoted H%[1]. Previous workers have examined NR/BR blend crosslink densities using DSC and the depression of solvent freezing points within swollen networks[5,6] but the two papers did not give consistent results. More recently Shershnev and co-workers have studied NR/IR blends using the swollen-state NMR technique[7].

'Network visualisation', a transmission electron microscopy (TEM) technique developed recently[8], was used in order to complement the NMR studies. The method involves swelling elastomer networks in styrene

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to equilibrium. The extent to which the network swells is dependent on its crosslink density. The styrene is polymerised and the elastomer component stained with osmium tetroxide for viewing by TEM. The images obtained show a dark mesh structure which is believed to be stained bundles of network chains within a light phase of polystyrene. The average mesh size is related to crosslink density as determined by standard physical crosslink density measurements[8]. A tightly knit mesh structure indicates a high crosslink density and a more open structure indicates a low crosslink density.

In this study the changes in crosslink levels throughout cure, for semi-efficient vulcanisation (semi-EV) sulphenamide accelerator cured natural rubber (NR), *cis*-1,4 poly(butadiene) (BR) and semi-EV and conventionally (CV) cured NR/BR blend vulcanisates with three different sulphenamide accelerators, are examined using the NMR technique.

EXPERIMENTAL

The rubbers used in this study were BR [Europrene Neo-*cis* BR40, 98% *cis*-1,4 poly(butadiene), Enichem] and NR (SMR-CV60). Rubber chemicals were standard commercial grade materials, and solvents were of AR grade except for the NMR solvents which were of spectroscopic grade [chloroform ($CHCl_3$), deuterochloroform ($CDCl_3$) and tetramethylsilane (TMS), Aldrich Chemical Company}.

Compounding was performed by mixing single polymers and blends in a BR-size Banbury internal mixer to the formulations in *Table 1* and the conditions in *Table 2*; curatives were then added on a two roll mill, *Table 3*. Cure times were determined using a Monsanto MDR2000E rheometer at 150°C. Cure times are expressed in terms of the time required to attain a specified percentage of torque rise from the minimum torque; thus t_{50} is the time at which 50% of the total increase in torque is achieved. Vulcanisates were prepared to times varying between t_{30} and twice t_{max} by programming the rheometer to cure to the appropriate time and retaining the rheometer disc. To ensure no further curing occurred, the vulcanisate was rapidly removed from the platens and the curing quenched by immersing the disc in iced water.

Vulcanisates were stored in a freezer at –26°C and analyses were performed as soon as practically possible to ensure no further curing.

The equilibrium volume swelling of single polymer vulcanisates in $CHCl_3$ was performed at 23°C; the volume fraction of swollen rubber in the gel, v_r, was calculated by the published method[9].

Physical crosslink densities of the single polymer vulcanisates were calculated from the Mooney-Rivlin constant C_1 determined from stress-strain experiments[10]. Physical crosslink densities in the blends are calculated from H% values determined from NMR spectra.

Small slivers of vulcanisates for NMR analysis were swollen in $CHCl_3$ at 23°C for 24 h. The $CHCl_3$ was refreshed twice to perform a cold extraction in order to remove low molecular weight material. This cold extraction is required because hot extraction of vulcanisates prepared to times before rheometer maximum (pre-t_{max}) would further cure the samples, invalidating analysis. The $CHCl_3$ was then solvent exchanged for $CDCl_3$ containing TMS as an internal lock; remaining $CHCl_3$ served as an internal marker.

TABLE 1. MIX FORMULATIONS

Compound	NR1	BR2	NR/BR3	NR/BR4
SMR-CV60	100	-	50	70
BR	-	100	50	30
Zinc oxide	5	5	5	5
Stearic acid	2	2	2	2
TMQ[a]	2	2	2	2

[a] Poly-2,2,4-trimethyl-1,2-dihydroquinoline.

TABLE 2. BANBURY MIX CYCLE[a]

Time (min)	Procedure
0	Add polymer/polymers
1	Add powders
2	Sweep
4	Dump

[a] Rotor speed = 116 r.p.m. Water flow rate varied to maintain temperature below 130°C.

^{1}H FT-NMR spectra were obtained using a General Electric QE300 300MHz Fourier-Transform spectrometer fitted with a ^{13}C/^{1}H dual 5 mm probe, Nicolet 1280 processor, and an Oxford Instruments 7 tesla super-conducting magnet, the data acquisition conditions of which have been reported previously[4]. The free induction decays (FIDs) were transferred to an Epson AX3S PC for manual phasing of the transformed FID, and the data were then transferred to a Prime minicomputer for further analysis. Peak widths in the ^{1}H NMR spectra were estimated by the parameter H%, which was determined using the reported method[1], but employing peak reference offsets of –0.2 p.p.m. and +0.1 p.p.m. from the olefin peak positions of NR and BR, respectively. Duplicate, or sometimes triplicate, spectra were obtained from freshly swollen samples.

Vulcanisates were prepared for the 'network visualisation' technique by following the published procedure[8]. Ultra-thin sections of the rubber/polystyrene composite were cut with freshly cleaved glass knives by using an RMC MT-7000 ultramicrotome and RMC CR-21 cryo-preparation unit operating well below the T_g of the BR phase. Specimens were collected on nickel examination grids and stained in osmium tetroxide vapour for two hours prior to examination with a Phillips EM300 TEM operating at 100 kV.

RESULTS AND DISCUSSION

The requirements for the analysis of crosslink density of a binary blend by swollen state NMR are calibration curves which relate n_{phys} to H%, for both single polymer phases of the blend, and those which relate the contributions of one polymer to the signal at the peak (P%H) and reference (R%H) positions of the other polymer to H%[1].

The calibration curves correlating H% with crosslink density from stress-strain measurements, accumulated from numerous single polymer NR and BR gum vulcanisates, are shown in *Figures 1* and *2*. The vulcanisates have base formulations NR1 and BR2, respectively, as given in *Table 1*. Over 40 different accelerated sulphur cure systems

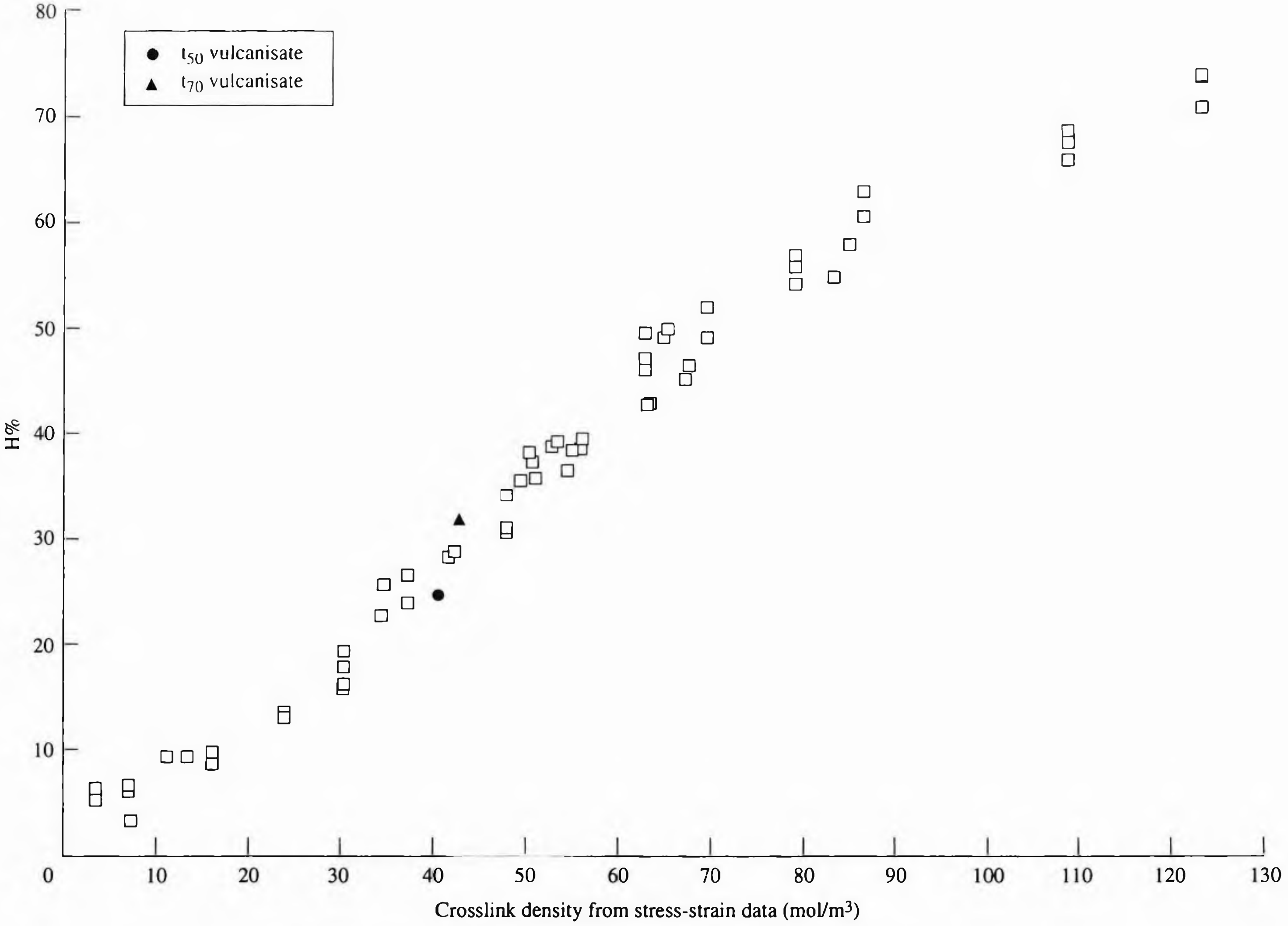

Figure 1. Dependence of H% on crosslink density for single polymer NR vulcanisates.

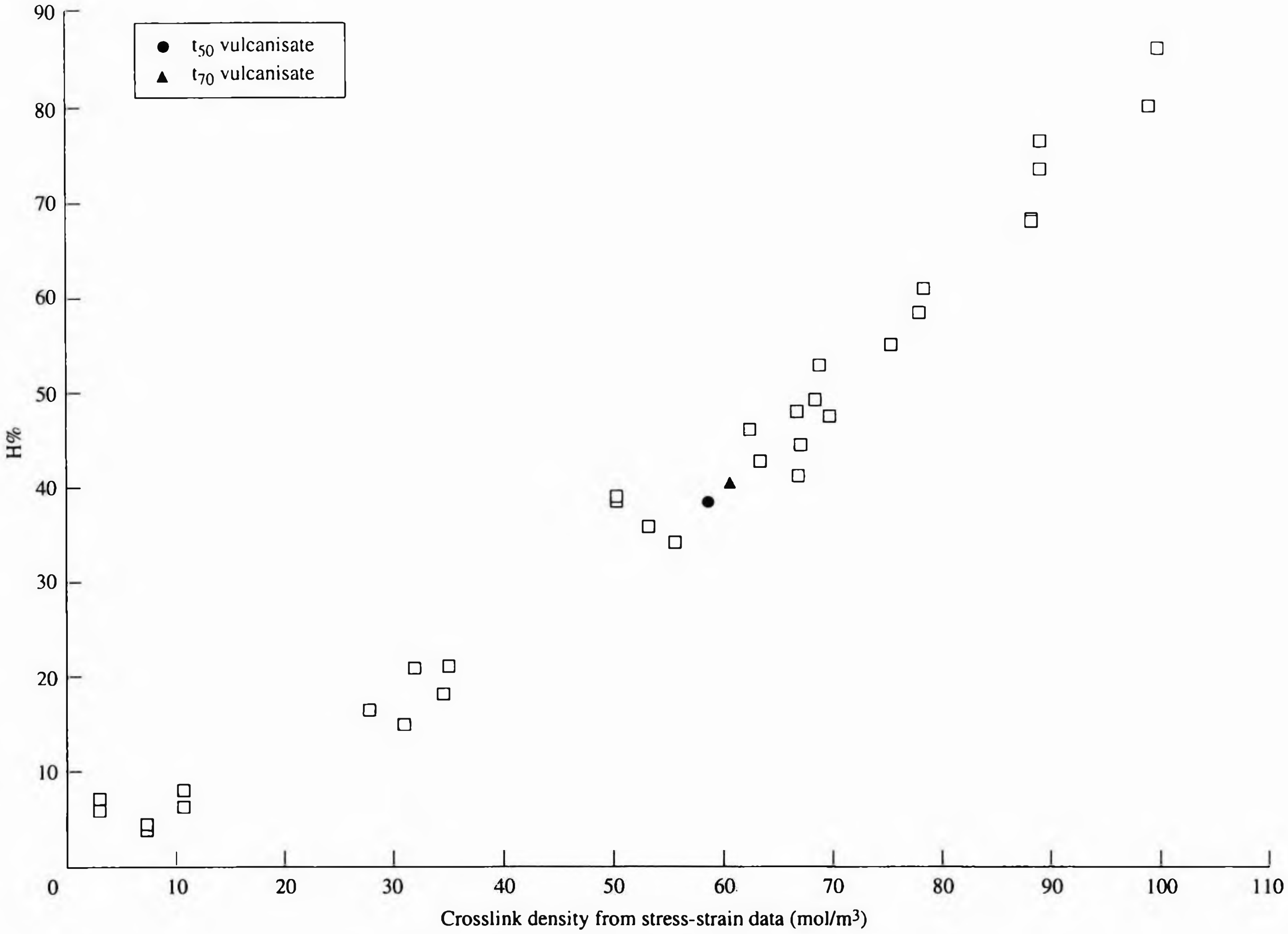

Figure 2. Dependence of H% on crosslink density for single polymer BR vulcanisates.

TABLE 3. CURATIVE LEVELS (p.h.r.)

Compound	NR1, BR2, NR/BR3			NR/BR4		
	A	B	C	A	B	C
Sulphur	1.2	1.2	1.2	2.5	2.5	2.5
CBS[a]	1.2	-	-	0.6	-	-
MBS[b]	-	1.2	-	-	0.6	-
TBBS[c]	-	-	1.2	-	-	0.6

[a]N-cyclohexylbenzothiazole-2-sulphenamide

[b]N-oxydiethylenebenzothiazole-2-sulphenamide

[c]N-*t*-butylbenzothiazole-2-sulphenamide

are included which range from conventional through to efficient containing either thiuram or sulphenamide accelerators. *Figures 3* and *4* show the correlation between H% and v_r for NR and BR, respectively. *Figures 1* to *4* show curves with a narrow band of scatter (3–4H%) which correlate physical parameters with H%. These observations are consistent with previous work which showed that H% is independent of the type of accelerated sulphur cure system used[2].

Because NMR peak width, and therefore H%, depends inversely on the mobility of the polymer-chain, there is the possibility that pre-t_{max} vulcanisates may exhibit increased H% values (*i.e.* decreased mobilities) due to accelerator pendant groups which act as crosslink pre-cursors. Pre-crosslink groups would also affect v_r values due to a change in the polymer-solvent interaction parameter (χ), but n_{phys} values calculated from stress-strain measurements should be less affected.

To ensure H% is not significantly affected, stress-strain and H% measurements were performed on NR and BR vulcanisates cured to rheometer t_{50} and t_{70}. Flat sheets 2 mm thick were compression moulded from compounds NR1A and BR2A and then quenched in iced water. Stress-strain and NMR data for these samples are indicated in *Figures 1* and *2* and fit the calibration curves generated from fully cured vulcanisates. This implies that any decreased mobility caused by pendant groups is not sufficient enough to affect the relationship between H% and n_{phys}. Crosslink densities for rheometer disc samples and blends, on which no stress-strain measurements are possible, are therefore calculated from the H% against n_{phys} calibration curves.

The spectra of single polymer gum vulcanisates were analysed to determine values for the parameters P%H and R%H, which are the terms used to correct for the overlap of peaks in spectra of blend vulcanisates[1]. Multiple linear regression indicates that P%H and R%H are correlated to H% by the following polynomial equations:

for the NR olefin peak at the BR peak and reference positions;

$$P\%H = -2.72 + 0.952H\%$$

$$R\%H = -0.74 + 0.476H\% + 0.0039H\%^2$$

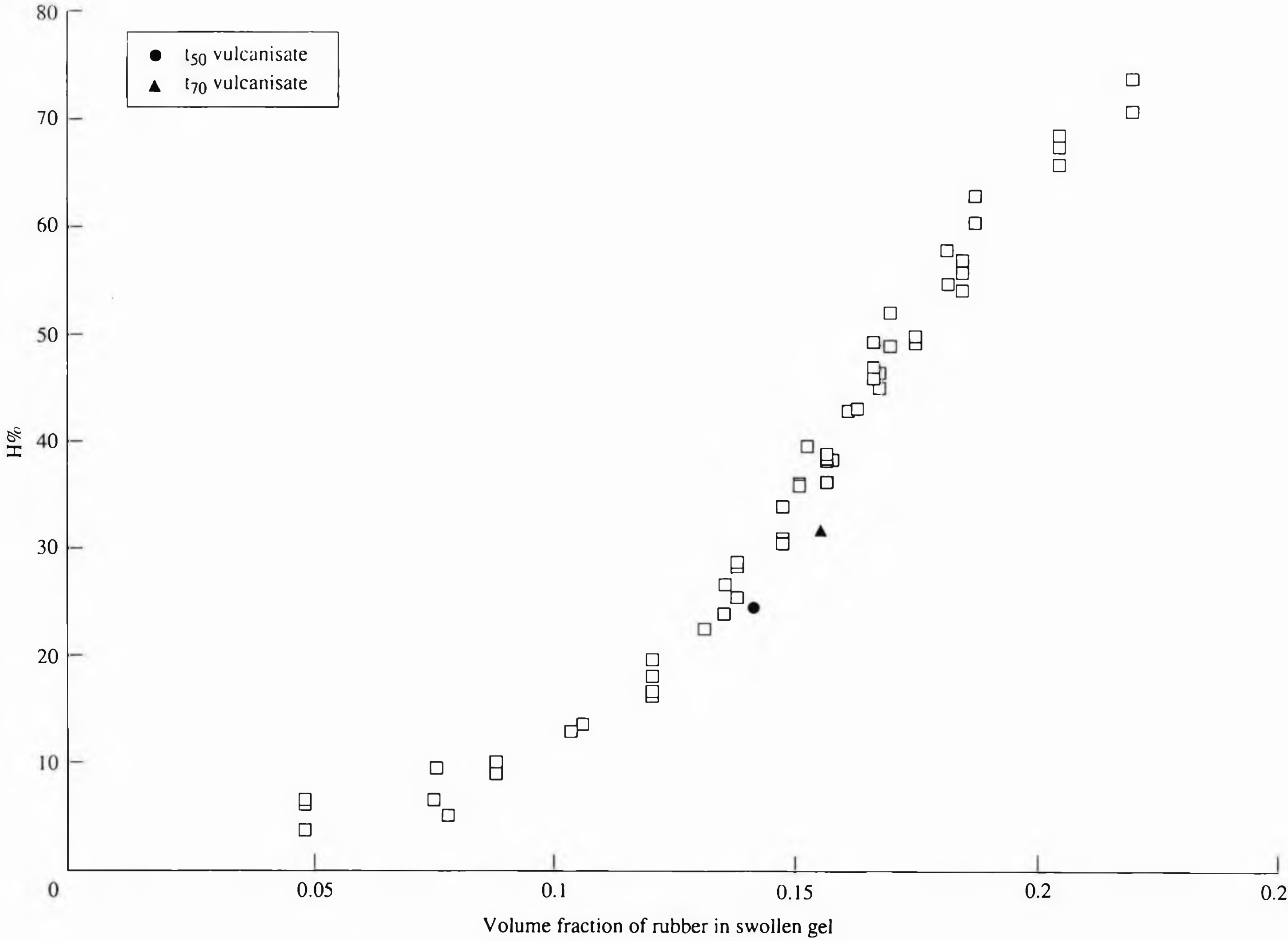

Figure 3: Dependence of H% on v_r *for single polymer NR vulcanisates cured to* t_{max}.

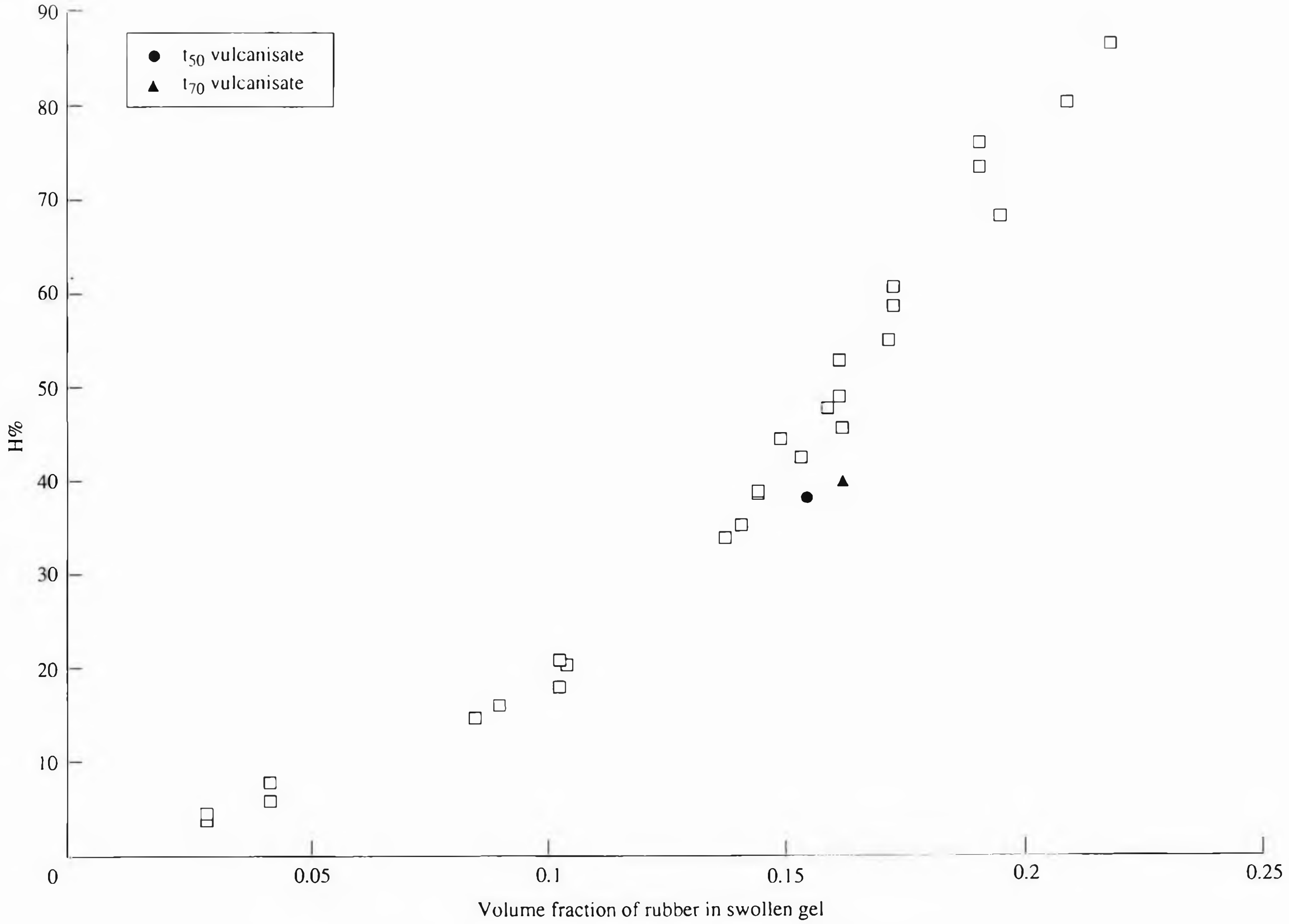

Figure 4. Dependence of H% on v_r for single polymer BR vulcanisates cured to t_{max}.

for the BR olefin peak at the NR peak and reference positions;

$$P\%H = 1.39 + 0.283H\% + 0.00582H\%^2$$

$$R\%H = 0.39 + 0.168H\% + 0.00557H\%^2$$

These equations are used in an iterative procedure to determine the H% values of each phase in blend vulcanisates[1]. H% may then be converted to crosslink density using the appropriate calibration curve.

The differences throughout cure for three different sulphur-sulphenamide combinations were studied in both single polymer and blend vulcanisates for semi-EV cure systems and in blend vulcanisates only for conventional cure systems. The accelerators considered were: N-cyclohexylbenzothiazole-2-sulphenamide (CBS), N-oxydiethylenebenzothiazole-2-sulphenamide (MBS) and N-*t*-butylbenzothiazole-2-sulphenamide (TBBS).

Single Polymers

Dependences of rheometer torque, v_r and n_{phys} (from H%) on cure time for NR vulcanisates are shown in *Table 4* and those for BR in *Table 5*. For both NR and BR, v_r data were found to fit the relationship with H% only at t_{max} (see *Figures 3* and *4*, respectively). This is expected because χ is known to vary with state of cure[11] and thus affects volume swelling. *Figure 5* shows the dependence of crosslink density (from H%) and v_r on cure time for sample NR1A. The figure shows that v_r attains high values at around t_{90} and maintains these high values on overcure, forming a plateau region. This illustrates the point that volume swelling measurements may not be used as a reliable indication of crosslink density under all cure conditions.

The values of n_{phys} (from H%) for the single polymers indicate that NR has shorter scorch times than BR and this confirms rheometric observations. At its maximum crosslink density, BR has crosslinked to a greater extent than NR with equivalent curative levels. Crosslink densities at t_{max} are similar for each single polymer regardless of accelerator and for both polymers the rate of cure is slowest with MBS accelerator as expected[12]. The data also indicate that for both polymers and all accelerators the maximum crosslink density as measured by H% is observed at times shortly prior to t_{max} generally t_{95}. Other workers[13,14] have found vulcanised physical properties, such as tensile strength, are also greater prior to t_{max}. These observations may be due to thermal lag of rheometer equipment, as suggested in reference 13, but may also be due to the method of the assessment of crosslinking. The stress-strain and swollen-state NMR measurements are performed at or close to ambient temperatures, whereas rheometry is performed at elevated temperatures. The possibility of interactions capable of acting as effective crosslinks under one set of conditions, but not the other, arises.

Blend rheometer torque values are influenced by the polymer ratio; a 70:30 NR:BR blend is likely to show torque values more indicative of the continuous phase. The BR phase may be highly cured but not influence the overall torque to a great extent because it is contained in a lower modulus matrix. For this reason rheometer torque values for blend materials may not be used as an indication of the state of cure.

TABLE 4. CROSSLINK DENSITIES OF NR VULCANISATES THROUGH THE CURE

Cure time (min)	Cure state	Torque (dNm)	V_r	H%	n_{phys}[a] (mol/m^3)
		NR1A, S/CBS			
9.0	t_{30}	1.60	0.072	15.9	25.9
9.5	t_{50}	2.95	0.095	21.2	33.1
10.3	t_{70}	4.86	0.126	32.6	47.1
13.0	t_{90}	6.63	0.145	33.7	48.5
15.0	t_{95}	6.95	0.146	34.3	49.2
23.7	t_{max}	7.11	0.146	34.3	49.3
47.4	$2t_{max}$	6.84	0.147	31.1	45.2
		NR1B, S/MBS			
14.3	t_{30}	1.31	0.052	9.0	14.4
15.2	t_{50}	2.31	0.075	18.5	29.5
16.3	t_{70}	4.24	0.101	28.6	42.2
19.7	t_{90}	6.33	0.137	36.6	52.0
22.0	t_{95}	6.80	0.139	41.5	54.0
33.0	t_{max}	7.34	0.152	35.4	50.5
66.0	$2t_{max}$	6.63	0.151	33.1	47.7
		NR1C, S/TBBS			
12.1	t_{30}	3.67	0.096	20.1	32.1
13.6	t_{50}	4.65	0.111	35.7	50.9
16.4	t_{70}	5.87	0.130	35.1	50.1
18.3	t_{90}	6.70	0.141	35.1	50.2
28.8	t_{95}	6.63	0.138	42.5	59.5
57.6	t_{max}	7.01	0.143	37.6	53.2
115.2	$2t_{max}$	6.76	0.145	30.3	44.2

[a] calculated from H% values

TABLE 5. CROSSLINK DENSITIES OF BR VULCANISATES THROUGH THE CURE

Cure time (min)	Cure state	Torque (dNm)	V_r	H%	n_{phys}[a] (mol/m^3)
		BR2A, S/CBS			
30.8	t_{30}	4.20	0.134	-	-
32.1	t_{50}	6.19	0.156	33.4	48.5
33.8	t_{70}	8.17	0.168	43.0	60.5
38.7	t_{90}	10.15	0.169	51.0	69.4
42.0	t_{95}	10.64	0.168	51.4	69.9
55.0	t_{max}	11.14	0.164	45.2	63.3
110.0	$2t_{max}$	-	0.151	42.9	60.8
		BR2B, S/MBS			
50.5	t_{30}	4.25	0.142	-	-
54.4	t_{50}	6.24	0.149	4.2	6.2
59.0	t_{70}	8.23	0.155	17.4	28.6
68.6	t_{90}	10.22	0.164	46.3	64.4
74.0	t_{95}	10.71	0.163	53.2	73.4
91.0	t_{max}	11.21	0.162	51.4	69.9
182.0	$2t_{max}$	-	0.149	43.7	61.6
		BR2C, S/TBBS			
41.0	t_{30}	4.22	0.141	14.0	22.8
44.5	t_{50}	6.22	0.148	26.7	41.3
48.0	t_{70}	8.21	0.154	39.9	48.7
56.5	t_{90}	10.20	0.166	43.5	61.1
63.0	t_{95}	10.70	0.165	52.6	70.7
80.0	t_{max}	11.20	0.167	43.2	61.1
160.0	$2t_{max}$	-	0.154	38.4	55.7

[a] calculated from H% values

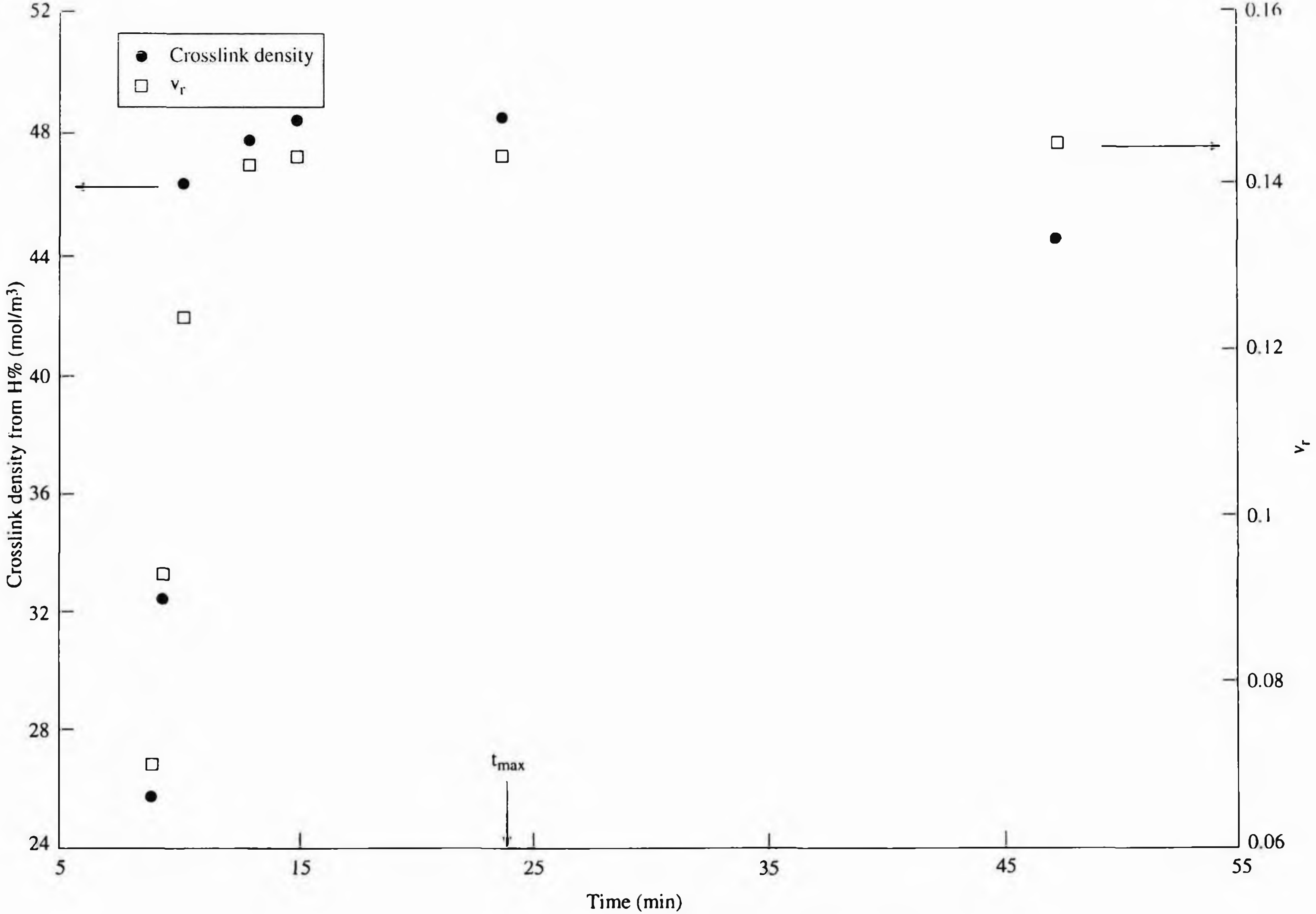

Figure 5. Dependence of crosslink density and v_r *for cure time for sample NR1A.*

Blends

Preferential location of accelerators within the phases of a blend occurs during mixing and their diffusion is rapid at vulcanisation temperatures; diffusion coefficients are in the range 10^{-7} to 10^{-8} cm^2s^{-1} for many curatives[15]. During the curing process the production of vulcanisation intermediates occurs, and these species will also move between the phases of blends in order to maintain their preferred distribution.

Rheometer torque and n_{phys} values (from H%) determined for NR/BR gum blend vulcanisates are shown in *Table 6* for 50:50 NR:BR blends with three differently accelerated semi-EV systems.

For all three systems the data indicate that at early stages in the cure the crosslink density of the BR phase is higher than in the NR phase. As the cure progresses the increasing difference in crosslink density of BR with relation to that in NR, indicates that initially the BR crosslinking rate is greater than the NR. Shortly after t_{50} (or t_{70} in the case of MBS accelerator) the differences in crosslink density begin to decrease, implying the relative crosslinking rates are reversed and NR is now crosslinking more rapidly. At t_{max} there is however still a bias of crosslinks in favour of the BR phase. *Figure 6* shows these effects more clearly for the S/MBS system. Note that the crosslink density value for the BR phase at t_{90} in sample NR/BR3A may be slightly high; the total crosslink density achieved in this blend exceeds that expected from the single polymers. This is not the case for the other systems.

If, in blend systems, no partition of curatives or related species occurs, then rheometer scorch times suggest that in blends the NR phase should be the first phase to cure. As the data indicate however, in blends the BR phase is the first to cure and at a faster rate than it does as a single polymer. The partitioning of sulphur between the phases of NR/BR blends is known to favour the BR phase by a factor of around 1.3 and accelerators also are more soluble in BR[16]. This suggests that diffusion of curatives and vulcanisation intermediates occurring throughout cure has the effect of delaying the onset of cure in the NR and enhancing it in the BR. The maximum crosslink densities attained are, however, close to those achieved in the single polymers. This implies that as the cure progresses there is a further movement of vulcanisation intermediates to redress the balance back to the NR phase with the net effect giving similar levels of crosslinking to the single polymers.

The rheometer scorch times of the 50:50 blends are not presented here but are approximately twice those of the single polymer NR. This suggests that some depletion of the natural activator/accelerator species present in the NR phase has occurred; migrating to the BR phase and shortening the vulcanisation induction period in the BR phase. The methylene carbons adjacent to the olefinic bond in BR are inherently more reactive than those in NR and thus once vulcanisation intermediates are produced they will react quickly with the BR.

Figure 7 shows the change in the ratio of BR:NR crosslinks (50:50 blend) with time for the three different accelerator systems. All three accelerator systems show high initial BR:NR crosslink ratios which decrease as the cure progresses. At maximum cure the BR phase is still more highly crosslinked than the NR phase

TABLE 6. CROSSLINK DENSITIES OF 50:50 NR:BR BLEND VULCANISATES THROUGHOUT THE CURE

Cure time (min)	Cure state	Torque (dNm)	NR n_{phys}[a] (mol/m^3)	BR n_{phys}[a] (mol/m^3)	Difference in n_{phys} (mol/m^3)	BR:NR ratio
			NR/BR3A, S/CBS			
18.4	t_{50}	4.72	21.4	57.3	35.9	2.7
19.5	t_{70}	6.39	44.3	65.0	20.7	1.5
23.5	t_{90}	8.08	49.2	77.8[b]	28.6[b]	1.6[b]
26.0	t_{95}	8.50	47.7	64.0	16.3	1.3
37.7	t_{max}	8.92	49.9	61.4	11.5	1.2
75.4	$2t_{max}$	-	47.7	53.8	-	1.1
			NR/BR3B, S/MBS			
32.2	t_{30}	3.03	<1[c]	12.3	11.3	12.3
34.1	t_{50}	4.68	2.0	23.0	21.0	7.6
36.3	t_{70}	6.33	38.4	67.1	28.7	1.7
41.3	t_{90}	7.97	52.4	78.4	26.0	1.5
44.2	t_{95}	8.38	50.4	71.4	21.0	1.4
57.9	t_{max}	8.81	48.0	65.9	17.9	1.3
115.8	$2t_{max}$	-	55.9	47.8	-	0.9
			NR/BR3C, S/TBBS			
23.1	t_{30}	2.83	26.4	51.8	25.4	2.0
24.1	t_{50}	4.42	31.6	64.7	33.1	2.0
25.7	t_{70}	6.00	40.0	65.9	25.9	1.6
29.7	t_{90}	7.58	48.3	68.3	20.0	1.4
32.1	t_{95}	7.97	56.1	62.0	5.9	1.1
43.7	t_{max}	8.37	46.4	56.3	9.9	1.2
87.4	$2t_{max}$	-	53.7	42.0	-	0.8

[a] Calculated from H% values

[b] BR n_{phys} value possibly in error, see text

[c] Beyond range of calibration curve

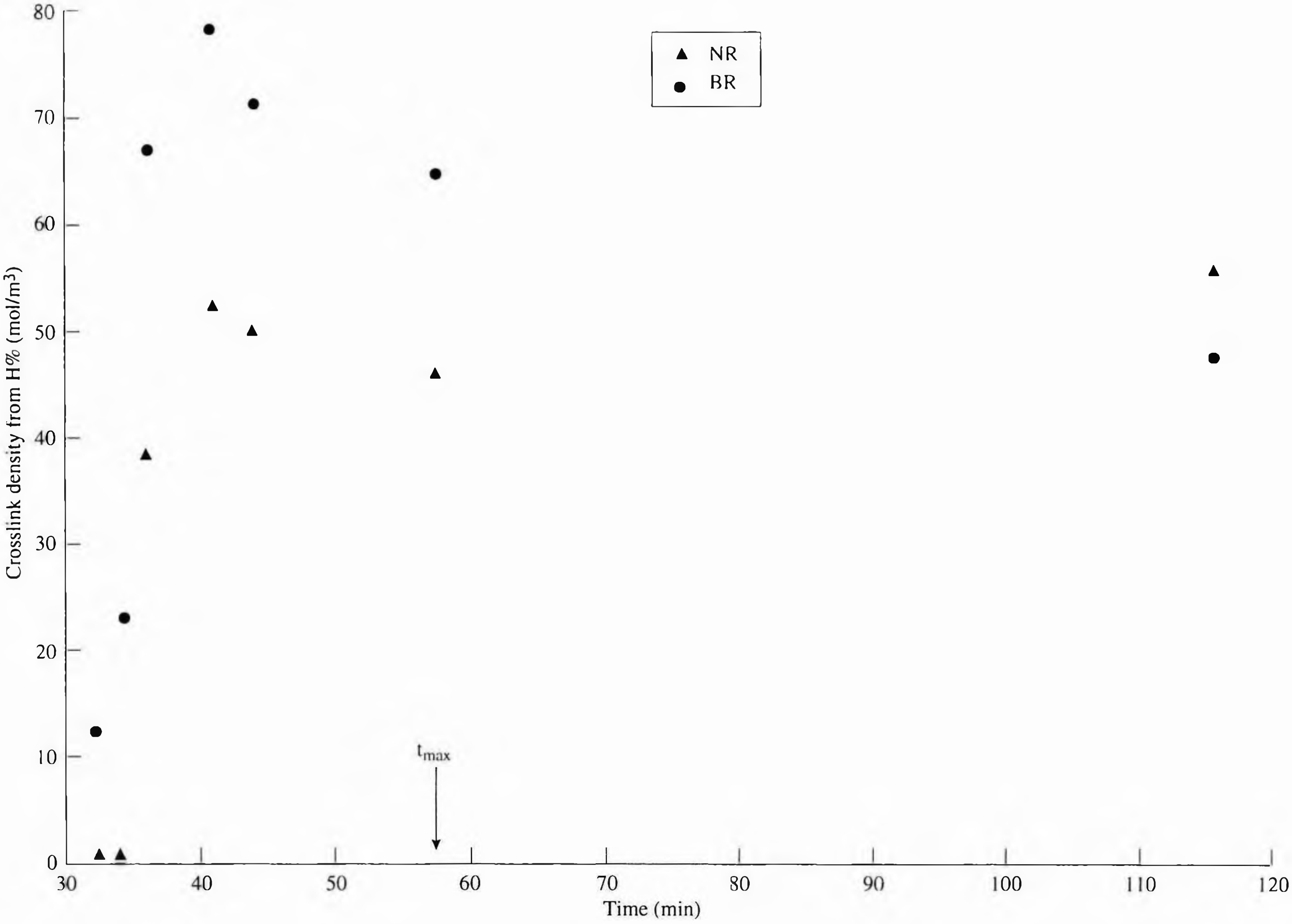

Figure 6. Dependence of crosslink density on cure time for both phases of sample NR/BR3B (50:50 blend, S/MBS cured).

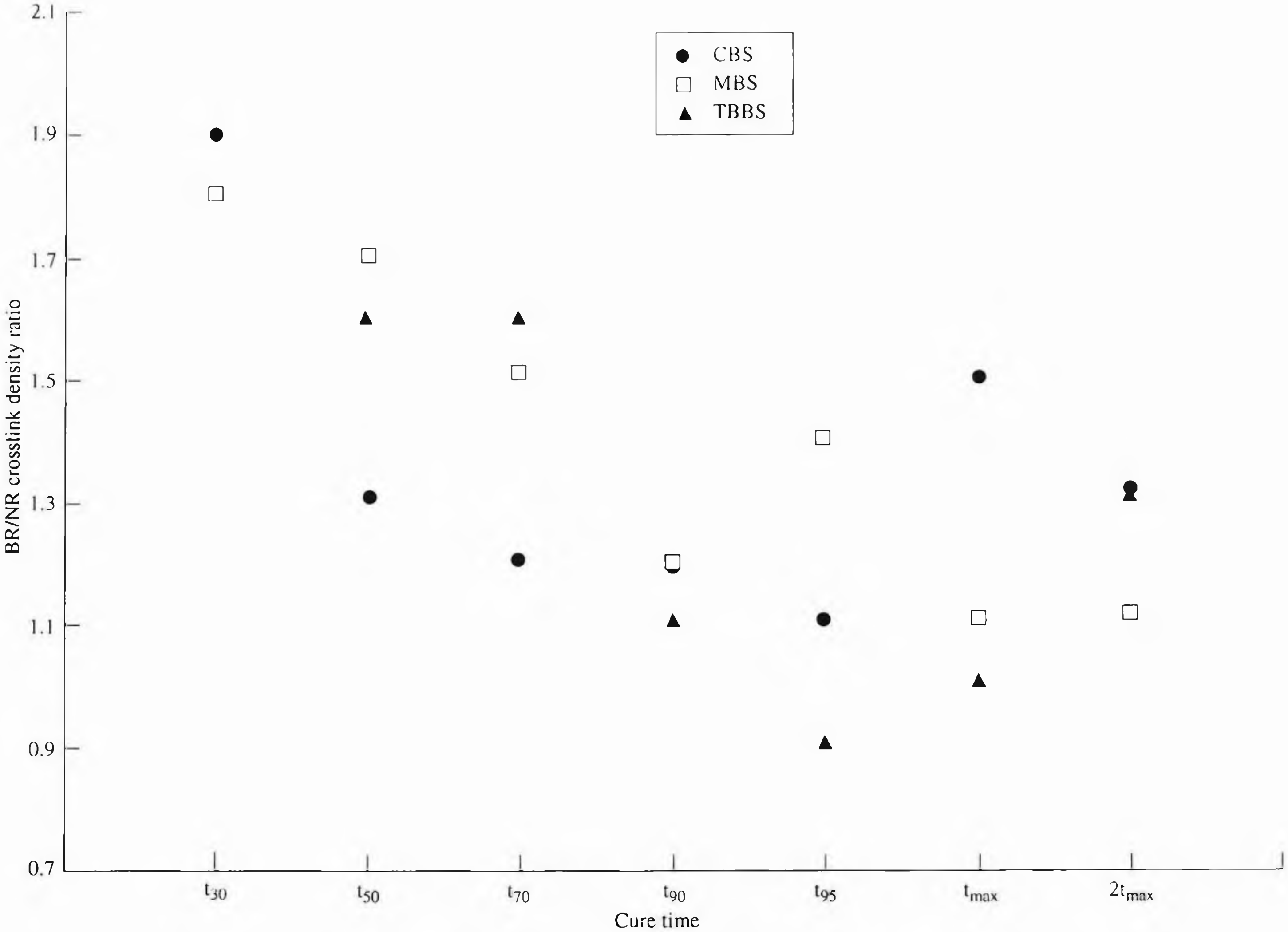

Figure 7. Variation of BR:NR crosslink density ratio on cure time for NR:BR 50:50 blends.

and the crosslink densities are very similar to those found for the single polymers with identical cure systems. McGill and co-workers used DSC either alone[5] or in combination with solvent swelling[6] to investigate 50:50 NR:BR and IR:BR blends and also found a higher crosslink density in the BR phase compared to that in the NR or IR phase.

Data for blends with a higher NR content (70:30) and vulcanised with conventional sulphur cure systems are presented in *Table 7*. *Figure 8* shows the variation of crosslink density (from H%) within the phases throughout the cure for the S/MBS system. The observations are similar to those for the 50:50 blend; crosslink densities are higher in the BR than the NR phase at maximum cure, and higher crosslink densities are attained in the BR at an early stage. One slight difference is that the BR:NR crosslink ratios are generally lower for 70:30 blends. This is seen more clearly by comparing *Figures 7* (50:50 blends) and *9* (70:30 blends) which depict the change in crosslink ratio with time. The difference may be due to the lower levels of accelerators in the 70:30 blends and this supports the suggestion that the partition of accelerator species in favour of BR is an important factor.

Shersnhev[7] and co-workers have investigated 70:30 polyisoprene(IR):BR blends by using the swollen-state NMR technique to study crosslink formation throughout the cure. Higher crosslink densities were reported for the BR phase of fully cured materials than in this work. The workers did not find that BR is the first phase to start curing in the blend system. This may be due to the differences between NR and IR. IR does not contain the natural activator/accelerators that are present in NR and thus movement of such species to enhance the BR crosslinking may not occur.

In order to verify that BR is the more highly crosslinked phase at short cure times a cured sample was studied[8] using 'network visualisation'. *Figure 10* is a micrograph of a swollen/polymerised stained section from sample NR/BR4A cured to t_{30}. The dark mesh structure is stained polymer network and the light region the unstained polystyrene. The micrograph shows quite clearly that there is a large difference in the mesh structure of the two phases. Morphologically NR:BR 70:30 blends are comprised of discrete regions of BR within a continuous NR phase and when swollen, the BR phase will remain as discrete regions. *Figure 11* indicates that the discrete regions have the more tightly knit mesh. A tightly knit mesh structure indicates a high crosslink density and a more open structure indicates a low crosslink density, therefore the BR phase has the greater crosslink density. Thus the microscopy technique supports the NMR evidence in favour of a highly crosslinked BR phase in NR/BR blends at early stages of cure.

CONCLUSIONS

Swollen-state NMR spectroscopy may be used to assess NR, BR and NR/BR blend crosslink density formation throughout cure for different sulphur-accelerator cure systems.

Single polymer NMR data indicate maximum crosslink densities are achieved slightly prior to t_{max} as determined by rheometry. This is in agreement with other workers who have shown physical properties to peak shortly before t_{max}[13,14].

Blend NMR data indicate that, in the two phase system, BR initially crosslinks more rapidly than NR for all cure systems and this

TABLE 7. CROSSLINK DENSITIES OF 70:30 NR:BR BLEND VULCANISATES THROUGHOUT THE CURE

Cure time (min)	Cure state	Torque (dNm)	NR n_{phys}[a] (mol/m^3)	BR n_{phys}[a] (mol/m^3)	Difference in n_{phys} (mol/m^3)	BR:NR ratio
NR/BR4A, S/CBS						
9.7	t_{30}	3.19	19.2	37.1	17.9	1.9
10.8	t_{50}	4.78	30.3	40.1	9.8	1.3
12.2	t_{70}	6.35	39.1	46.7	7.6	1.2
15.4	t_{90}	7.93	50.4	59.6	9.2	1.2
17.2	t_{95}	8.33	59.3	63.9	4.6	1.1
23.8	t_{max}	8.73	50.5	73.6	23.1	1.5
47.5	$2t_{max}$	7.95	45.4	60.3	-	1.3
NR/BR4B, S/MBS						
10.4	t_{30}	3.18	10.7	19.5	8.8	1.9
12.0	t_{50}	4.71	21.9	36.7	14.8	1.7
13.8	t_{70}	6.24	36.1	55.3	19.2	1.5
17.2	t_{90}	7.77	50.9	63.4	12.5	1.2
19.1	t_{95}	8.15	51.5	70.2	18.7	1.4
26.3	t_{max}	8.53	55.0	62.2	7.2	1.1
52.6	$2t_{max}$	7.75	48.4	51.8	-	1.1
NR/BR4C, S/TBBS						
11.6	t_{30}	3.01	<1[b]	7.3	6.3	7.3
13.0	t_{50}	4.43	17.9	28.7	10.8	1.6
15.0	t_{70}	5.85	32.5	53.4	20.9	1.6
18.9	t_{90}	7.27	45.7	48.6	2.9	1.1
21.2	t_{95}	7.62	55.5	51.8	-3.7	0.9
29.6	t_{max}	7.98	50.9	48.4	-2.5	1.0
59.1	$2t_{max}$	7.13	38.1	51.2	-	1.3

[a] Calculated from H% values

[b] Beyond range of calibration curve

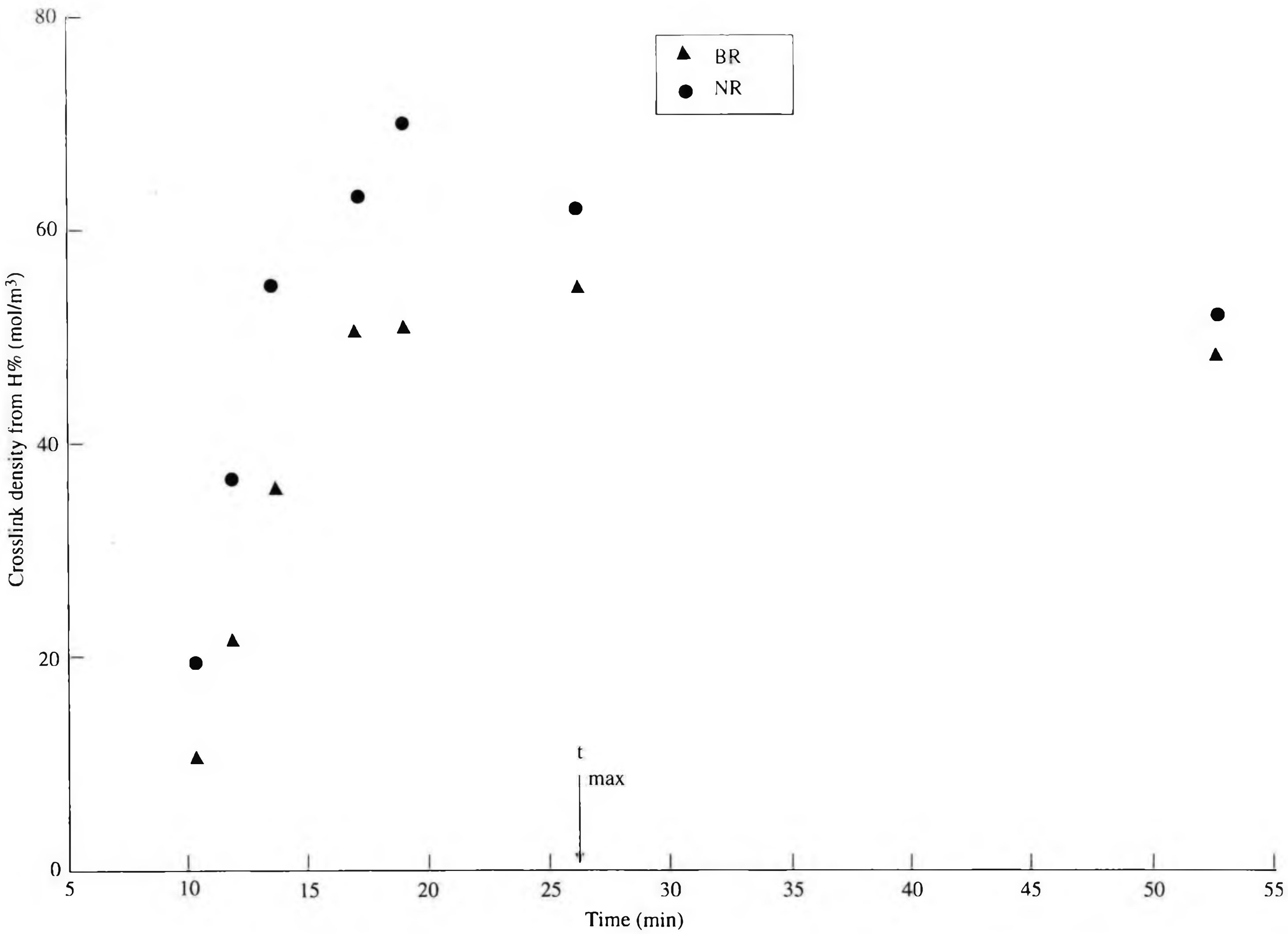

Figure 8. Dependence of crosslink density on cure time for both phases of sample NR/BR4B (70:30 blend S/MBS cured).

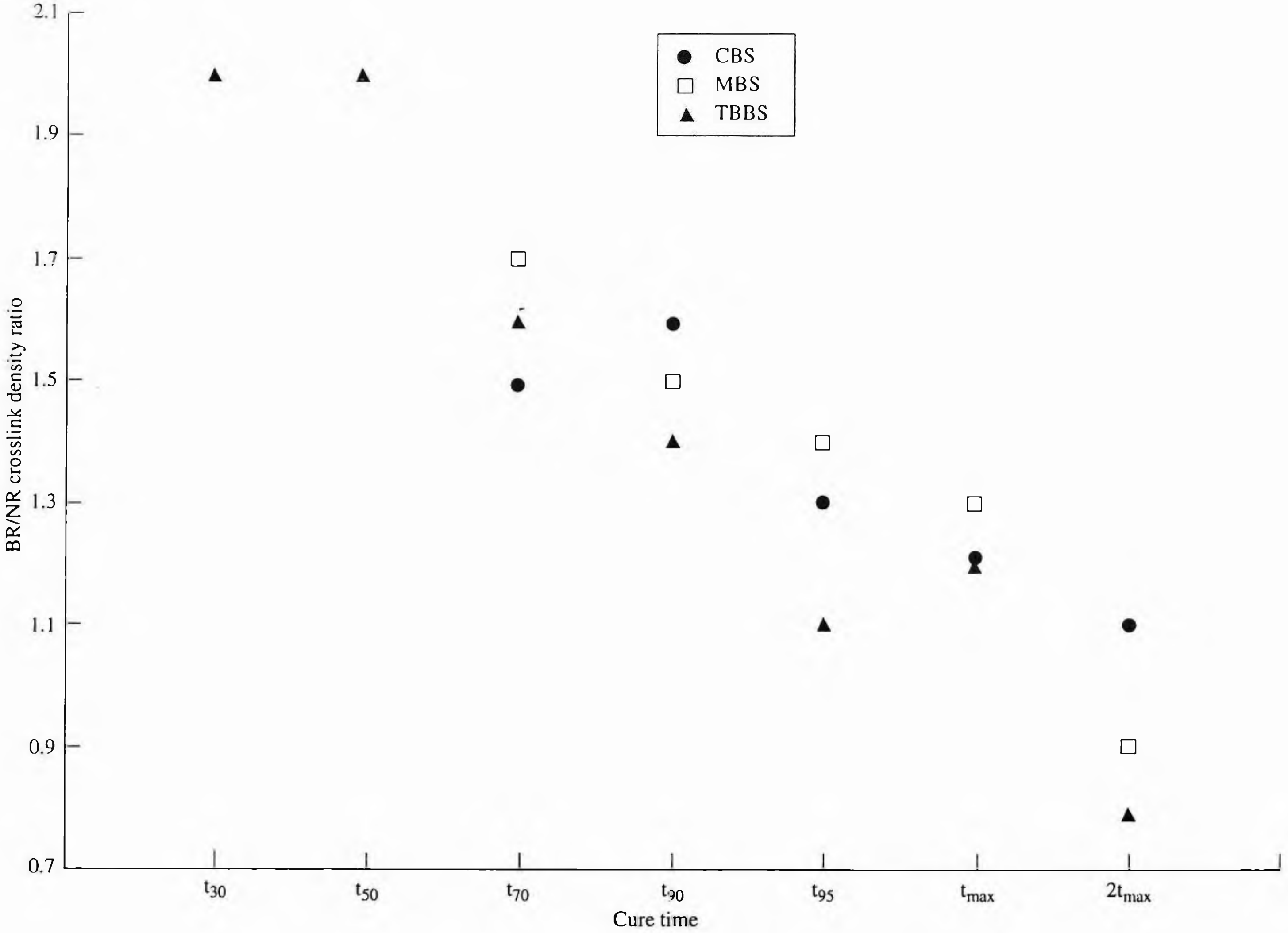

Figure 9. Variation of BR:NR crosslink density ratio on cure time for NR:BR 70:30 blends.

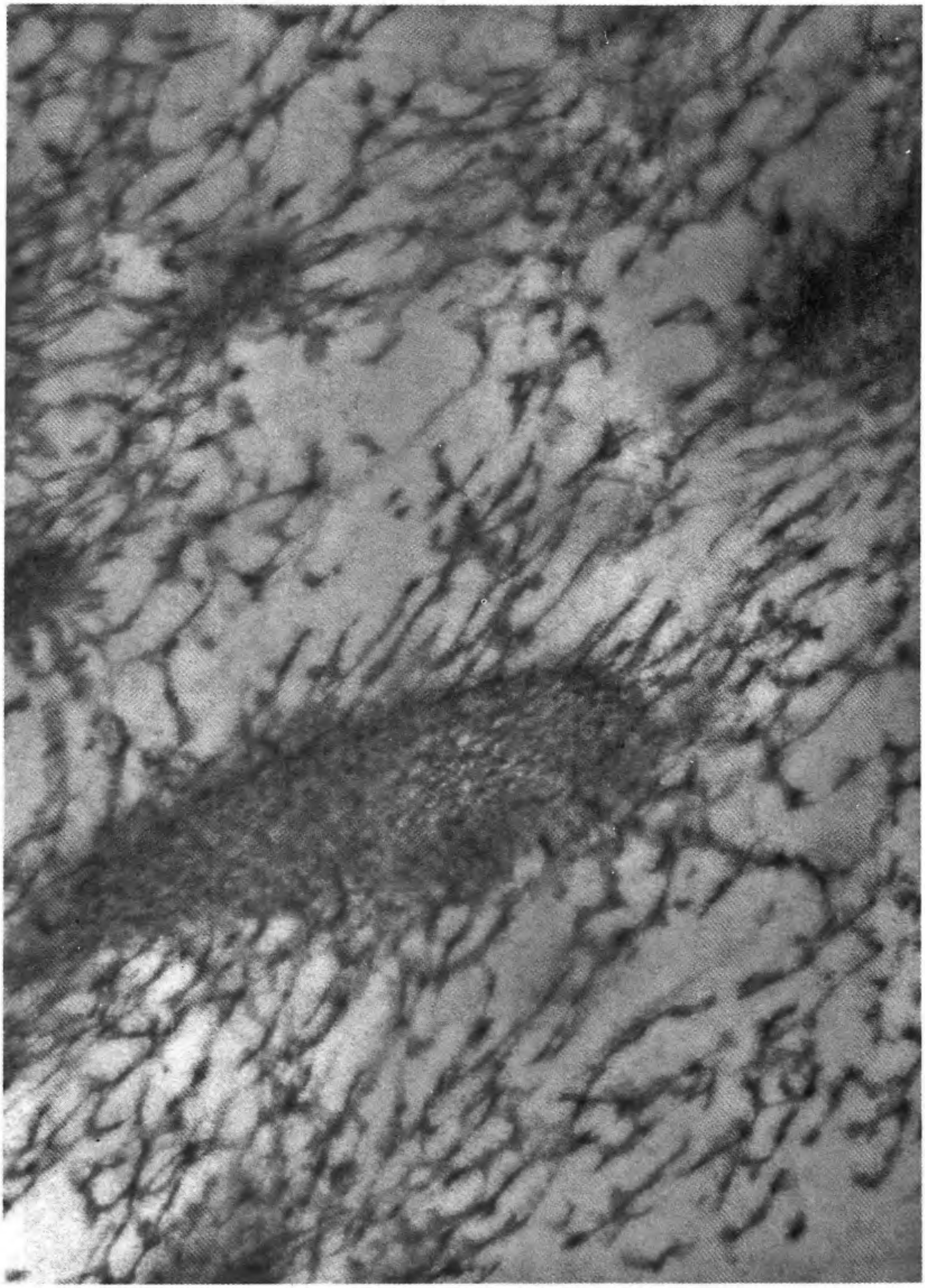

Figure 10. TEM micrograph of a swollen/polymerised t_{30} *sample of NR/BR4A indicating the network density is different for the two phases.*

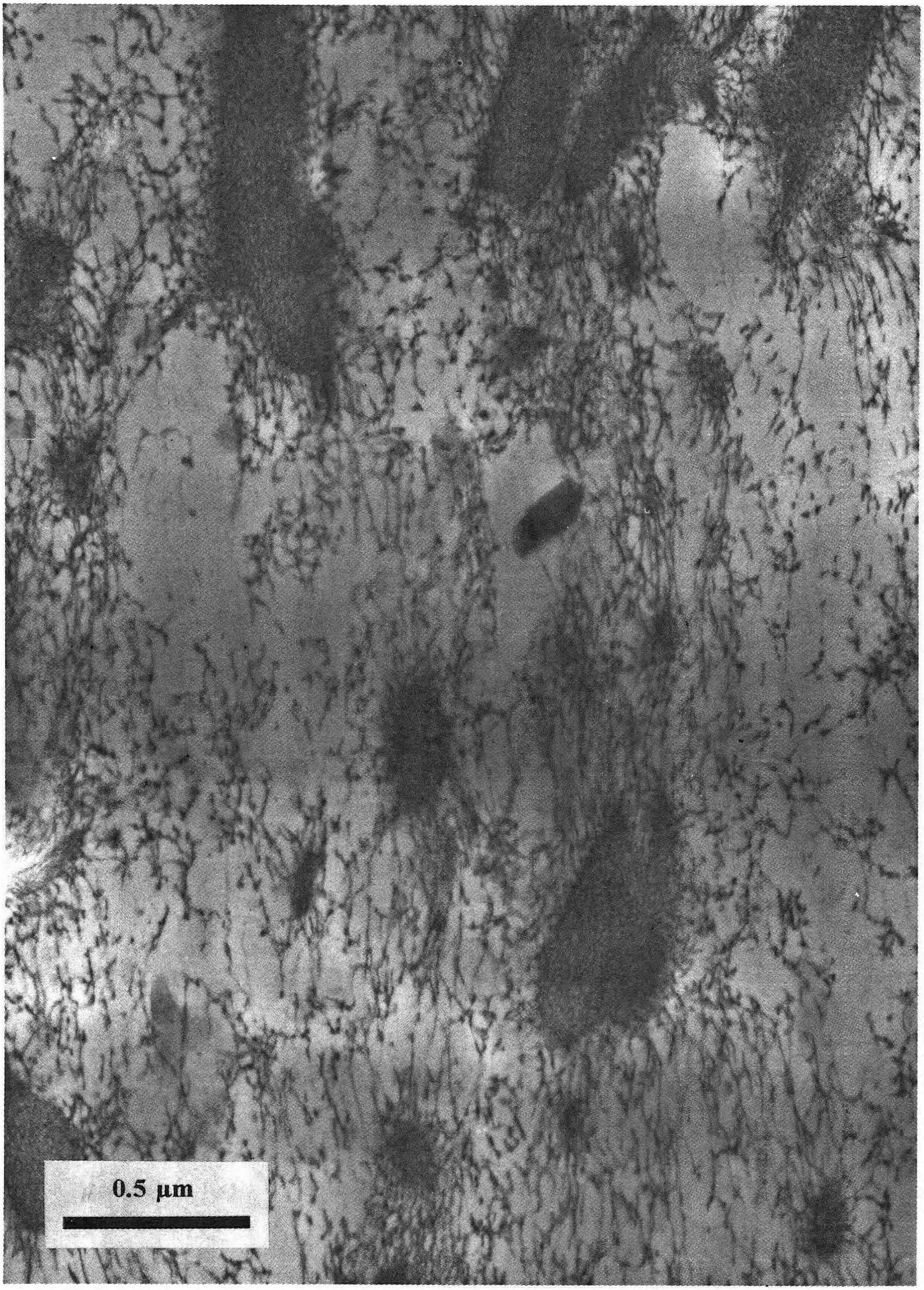

Figure 11. TEM micrograph of a swollen/polymerised t_{30} *sample of NR/BR4A showing the morphology of the 70:30 blend.*

has been confirmed by the 'network visualisation' technique. This may be due in part to increased partitioning of sulphur and accelerators in favour of the BR, but also to natural activators/solubilisers present in NR diffusing into the BR phase. Reducing the level of accelerator in the blends decreases the initial difference in crosslink density and supports the suggestion of curative migration. As the cure progresses in the blends the NR phase cures more rapidly than the BR and the initial large differences in crosslink density are reduced. At maximum cure BR is still the more highly crosslinked phase. Where comparison is possible the crosslink densities in the blend at maximum cure are comparable to those indicated by single polymers.

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Sectioning Techniques for Elastomer Blend Preparation by Ultramicrotomy for Transmission Electron Microscopy

P.E.F. CUDBY*

The preparation of samples for transmission electron microscopy has often been referred to as more of an art form than a science. This definition has arisen because of difficulties, on the part of microscopists, in defining rules by which the necessary ultra-thin sections can be prepared. Increasingly sophisticated apparatus has alleviated some of the problems related to reproducibility but it has been observed that different operators still continue successfully to use different conditions to achieve the same results. Elastomers are considered by many to be one of the most difficult groups of materials to prepare for electron microscopy. This paper describes some of the techniques involved in sectioning such materials and some of the special conditions that can be applied to improve the chances of success. Many working in this field will have come from a biological background and so consideration is made of the differences in preparation and examination between biological materials and elastomers. Examples of micrographs taken of sectioned elastomer blends are given.

Numerous light microscopical techniques exist for the examination of blend morphology[1,2,3]. However, all of these suffer from the same drawback; the maximum attainable resolution available using a visible light source is physically limited to approximately 0.25 µm by the wavelength of visible light. Consequently in some materials it becomes difficult to observe blend morphology and it is generally impossible to observe any micro-structure within a phase (micro domains, filler location *etc.*). This is not to suggest that there is no place for light microscopy (LM) since clearly that is not so but, with the improvements made in blend technology and the ever-increasing demand for information, many laboratories have discovered that high resolution imaging of blends is no longer an expensive luxury but a necessary requirement. For the laboratory that finds itself in this predicament, only three options really exist:

- Convert an existing scanning electron microscope (SEM) to transmission imaging (the author has dealt with this possibility elsewhere[4]). This provides a dramatic improvement in resolution over light microscopy but does not compete with a transmission electron microscope (TEM) in high resolution terms.
- Set up a fully equipped transmission electron microscope suite.

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- Find a consultancy such as *Rubber Consultants* that offers these facilities and has experience with them.

It should be stated at the outset that good TEM imaging depends upon good specimen preparation. It therefore follows that poor quality specimens inevitably lead to poor TEM images. Unfortunately, there are no short cuts and if the facilities and manpower are not available to do the job to a sufficient standard then the laboratory in question would be well advised to send the necessary work to a third party. If the correct preparative instrumentation is not available, it is highly unlikely that reasonable specimens can be prepared.

The aim of this paper is to describe in some detail the preparation of elastomers for imaging by transmission electron microscopy and to give some examples of the kind of images that can be obtained. Comparisons will be drawn between the information available by LM and that available from identical samples by TEM.

SPECIMEN PREPARATION

The aim of this section is to provide some detail about the preparation of good quality thin and ultra-thin sections. The most easily interpreted images of phase morphology are usually obtained by transmission imaging of materials in cross-section. (Solvent casting of thin films of unvulcanised material is also possible but this topic is not considered here). The preparation of thin and ultra-thin sections of elastomers and elastomer blends is without doubt the most time-consuming aspect of microscopical examination. It must be reiterated that good micrographs can only be obtained from good sections and these can only be obtained by patience and taking the time to develop the necessary skills. In fact the actual taking of the micrograph often accounts for only a fraction of the time involved in the overall analysis. It should also be noted that the right equipment is essential, thus it can be argued that it is highly unlikely that reproducibly preparing ultra-thin sections for transmission electron microscopy using a base-sledge microtome with a steel blade would be possible. The laboratory at MRPRA is equipped with an RMC MT7000 Ultramicrotome with a CR-21 Cryo Prep. Unit. This instrumentation can maintain cryogenic temperatures to ±0.1°C and without this kind of thermal stability the preparation of ultra-thin sections would be more a matter of luck than any skill on the part of the operator.

At this point it is necessary to discuss how thin a usable ultra-thin section should be. In terms of obtaining an image it is unlikely that an operator with a 100kV TEM will be able to obtain meaningful images from sections that are thicker than 200 nm in a simple gum blend. However the usual statement that 'the thinner the better', meaning that if a section is greater than 50 nm thick it is of no use, is patently untrue. It would certainly be true to say that the thinner the section the higher the resolution image that can be obtained from that section, but very few examinations in this context require 5 nm resolution! It is therefore necessary to apply some common sense and an awareness of economics to the subject. Anyone with any understanding of the nature of the problems involved with sectioning elastomers will be aware that a general rule of thumb is that the thinner the section, the longer it will take to obtain the conditions required to obtain that section (and consequently the more it will cost in real terms to produce that section). Cosslett, however, added the rule that the maximum resolution obtainable was approximately one tenth of the thickness of the section[5,6]. Modern instruments have various techniques for correcting for chromatic blurring but Cosslett's figure still serves as a useful guide. This could be restated that the section could be up to ten times as thick as the resolution required, provided instrumental limitations are taken into account. Therefore, if the operator plans to use TEM for imaging at reasonably low magnification and requires a resolution of merely 20 nm then, in theory, the sections used could be up to 200 nm thick. Had the operator spent extra time trying to obtain sections that were 50 nm thick then it could be argued that the time could have been used more effectively. Common sense also dictates that if the morphological or structural features of interest are likely to be much smaller than the anticipated section thickness then there is a danger of overlapping features and confusion of detail. However, this may not necessarily be a problem provided that the operator is willing to spend some time interpreting the image. For example, the TEM micrograph of the NR/EPDM blend considered below is thick enough to reveal a great deal about the structure of the EPDM phase within the NR matrix although there is some confusion of detail regarding the micro domains. What is required therefore is a little thought as to what information is really required before preparation begins.

It should also be added that, contrary to much popular opinion amongst biologists, it is better to operate the TEM at high accelerating voltages to aid resolution, improve the brightness of the image and minimise beam damage. Traditionally it has been held that low accelerating voltages will improve contrast and give better images but this is not the case with elastomers. Most of the contrast in images of elastomers is artificially produced by chemical staining and further contrast can be added if necessary at the photographic printing stage. In fact, after micrographing a chemically stained blend it is often necessary to remove contrast at the printing stage because a photographic print does not have the contrast range that a TEM negative has. Failure to do so can result in areas on the print being totally white or black and therefore containing no information. The use of low accelerating voltages can also increase the rate at which polymers succumb to damage by the electron beam since the lower energy beam actually heats the specimen more than a higher energy beam to which the specimen is more transparent.

SECTIONING TECHNIQUES

The Knife

For ultramicrotomy there are really only two choices, a glass knife or a diamond knife. (There was a short-lived attempt at introducing sapphire knives several years ago but these do not appear to have attracted sufficient long-term interest to survive). The advantage of a diamond knife is that it is intrinsically sharper than a glass knife and the edge remains sharp for far longer. However, as might be expected, diamond knives are extremely expensive to buy and they have to be kept and used very carefully since they are easily damaged. The author has tried a number of diamond knives on materials filled with either zinc oxide or silica and in each case the knife edge was damaged quite quickly, presumably by individual particles within the matrix. It is not known whether all diamond knives will behave in this manner with filled elastomers but the knives tested were rejected on the grounds of lack of longevity. On the positive side, the sections which were cut were thinner and less prone to most artefacts than those cut using a glass knife. However knife marks were apparent and, as discussed below, knife marks are usually a clear sign of damage to the knife edge.

In contrast, glass knives are cheap to make and are disposable after use. It is true that it is unlikely that an operator will be able to produce a glass knife that is as sharp as a diamond knife, but from an economic point-of-view many laboratories cannot justify the costs of purchase and regular resharpening (usually about half the cost of the original knife) of a diamond knife. Glass knives are usually produced in-house by a dedicated knife making apparatus. A knife maker is a mechanical device for reproducibly scoring and breaking glass into knives. It should be stressed that making a glass knife can be a time-consuming process and that the best knives (with the fewest stresses in the edge) are made by a slow break usually taking in excess of fifteen minutes. Knives can, of course, be broken more quickly for LM where ultra-thin sections are not required. When broken, the knife edge has the appearance illustrated in *Figure 1*. A rule of thumb is that the slower the break, the fainter the stress mark. With practice it is possible to break knives in which the mark is almost invisible.

There are essentially two regions on the knife edge. The right-hand two thirds of the knife are generally unsuitable for ultramicrotomy due to increasing roughness. This part of the knife is best used for trimming the block face prior to sectioning. The left-hand third of the edge is far more regular and seems to become sharper towards the left. There is a tendency for the edge to become rough where the stress mark joins it but once again a slow break reduces this problem. Indeed there are a number of blends that can only ever be sectioned at the far left-hand side of the knife.

Sectioning Temperature

To consider sectioning temperature effectively, it is first necessary to consider the nature of the 'cut' itself. A simple attempt at cutting a vulcanisate with a razor blade illustrates the difficulties involved with cutting rubber. In order to section rubber, it is necessary to reduce its temperature to below its glass transition temperature (T_g). However, if natural rubber with a glass transition temperature of –72°C is considered, it is unlikely to be sufficient simply to reduce the temperature to –80°C. This is because when one is sectioning below the T_g of a material one is not actually cutting the specimen but fracturing it in a controlled manner. The process of fracturing the sample liberates energy in the form of heat which can raise the localised temperature substantially at the tip of the knife. The rise in temperature is likely to be dictated to some degree by other sectioning conditions including the sectioning speed, which is the speed at which the sample is passing over the knife. However, the important point to note is that it is quite possible to raise the temperature at the fracture initiation point to above the T_g. The effect of this would be that the knife may then have a tendency to stick into the sample causing a number of artefacts including tearing. Some operators have suggested that a localised temperature rise of 50°C is possible[7], but experience suggests that, provided the operator is not trying to section using 'impossible' conditions, it is necessary to be merely 20–30°C below the T_g. If the material under consideration is a blend it is usually important to judge the sectioning temperature from the lowest T_g.

Size and Shape of the Block Face

The size and shape of the block face are far more important than one might imagine. With

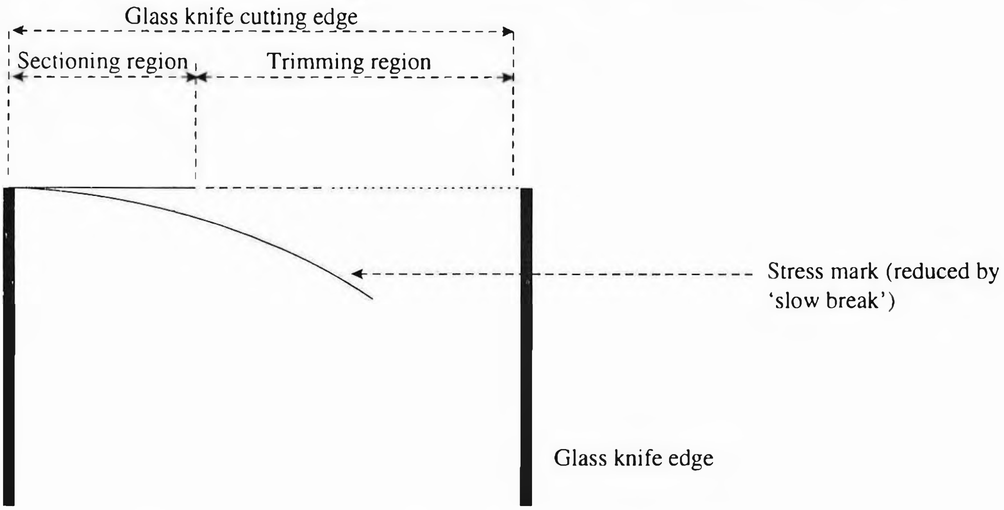
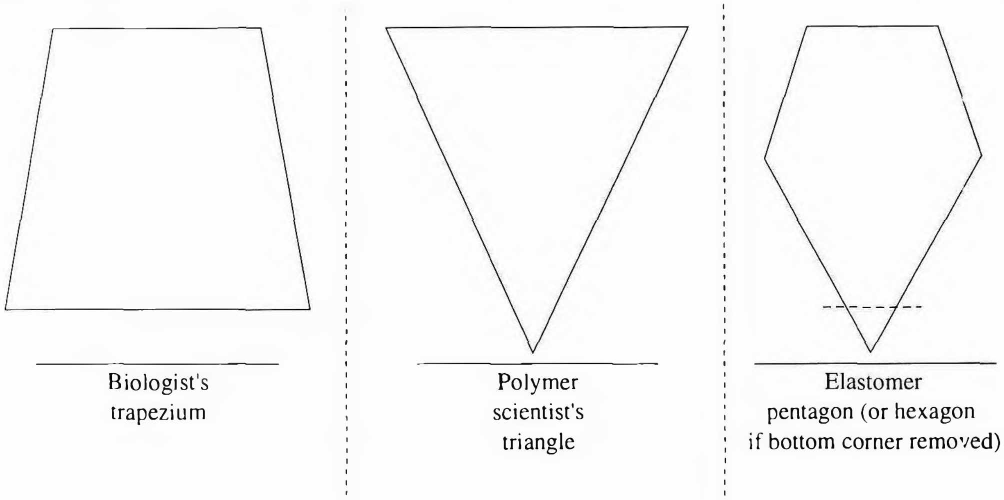


Figure 1. Diagramatic representation of a glass knife edge viewed from above.

respect to the size of the block face the general rule is 'the smaller the better' and it is suggested that the best results are usually obtained from block faces less than 0.5 mm wide with the longest face oriented vertically. In terms of shape, a large number of books have been written about microscopy and microtomy concentrating on biological specimens and much of the advice given does not apply to elastomers to the point that application of some of the ideas can be distinctly unhelpful. One such idea concerns the shape of the block face. Biologists generally examine materials which have been embedded in a hard polymer and can be sectioned at room temperature. Consequently they are able to section into a trough filled with water and can produce long ribbons of sections that float. Their preferred block face shape is illustrated in *Figure 2* which maximises the chance of ribbon forming. When one is sectioning at cryogenic temperatures this is not possible and neither is the trapezoid shape desirable with its broad leading edge often blunting the edge of the knife. Instead two other shapes are proposed; the first of these seems to be fairly common practice in polymer circles which is to use a triangular shaped block with the sharp edge pointed towards the knife. This is a useful shape but has as its greatest pitfall the problem of the section not detaching from the block at the end of the cutting cycle. A little consideration of the problem leads to the simple modification to the shape as shown in *Figure 2* which reduces the likelihood of the section not detaching. As with much of sectioning this is a simple change but it makes a big difference to the ease with which sections can be prepared. It is likely that the operator will experience problems in trimming the block to such a precise shape. The suggested technique is to trim the block face to a triangle using a razor blade prior to mounting it in the microtome. It should be cooled to the required temperature at which it is then possible to trim the block using a small pre-chilled scalpel.

Knife Angle, Clearance Angle and Sectioning Speed

These could be described as general operating conditions. It is the interplay between



Biologist's trapezium

Polymer scientist's triangle

Elastomer pentagon (or hexagon if bottom corner removed)

Figure 2. Different block face shapes.

these three sets of conditions that usually determine whether sectioning will be successful or not, provided that the correct temperature has been established. The distinction between knife angle and clearance angle should first be drawn. These are illustrated below in *Figure 3*.

When working with glass knives there is always a competition between their sharpness and longevity. Experience suggests that knives made with small knife angles, *e.g.* 35°, tend to be sharper than knives made with large knife angles, *e.g.* 60°. The choice of the knife angle is often reliant on the particular operator and the way in which he or she sections. Some dependence also exists on the type of specimen under consideration. If the sample is very hard then using a larger angled knife may be necessary. However, it is likely that this will be at the cost of sharpness and consequently the sections cut may not be of a desirable thickness. For most elastomer applications it has been found that a 45° knife seems to provide the optimum conditions. It should be noted that the actual knife angle may differ from the angle set on the knife maker since the fracture will tend to curl away towards the knife edge at the end of the score mark.

The clearance angle is determined by the hardness of the specimen at the temperature used in that the harder the block is, the larger the clearance angle will need to be in order for sections to be cut. This angle can be varied between 1° and 10° on most ultramicrotomes. However, it should also be noted that the steeper the angle, the more prone the sections will be to compression because the knife will have a tendency to 'stick in' to the block face. There is also a tendency for the knife to scrape across the surface of hard block faces if the clearance angle is too shallow. If this happens it will cause a rapid blunting of the knife edge, and this will be further degraded when the knife finally catches because the section will be far thicker than originally intended. It is therefore up to the operator to judge the specimen and set the clearance angle accordingly.

Sectioning speed is dictated by both the sample and the operator. No clear guidelines

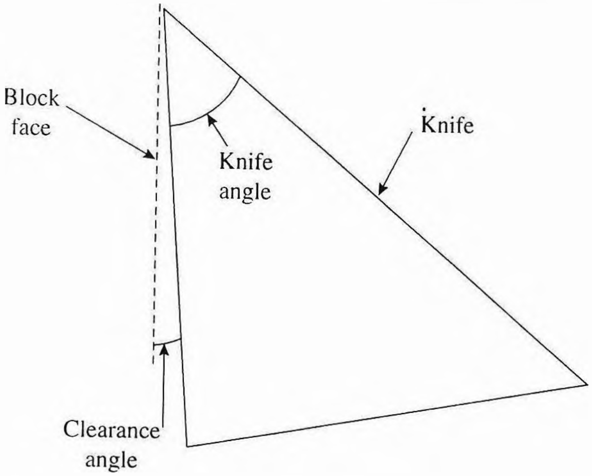


Figure 3. The knife angle and clearance angle on a glass knife.

can be set but it is often that sectioning speed dictates, more than any of the other variables with the possible exception of temperature, whether sectioning will be successful or not. Usually it is better to start at a slower speed and increase if necessary. With practice it becomes more easy to section manually since the operator maintains more control than if motor control is selected. The speed at which one chooses to section is very much a part of the 'art' of sectioning.

Sectioning Using a Trough Liquid

Again this is a point at which skills and practices developed for biological specimens are largely inapplicable to elastomers. As stated above, biologists usually work at room temperature with embedded samples. With such samples it is possible to section into a knife trough filled with water on to which the sections will float and from which they can be easily collected. However, when one is working at cryogenic temperatures, water is not a viable option as a trough liquid (except in the special case described below). There are some trough liquids available that do not freeze at the cryogenic temperatures described here, although sections do not float on these in the same way that they do on water at room temperature. One such example is n-propanol which is effective down to about −125°C. The best results can be obtained from having a small amount of n-propanol in the trough that can be swept carefully up the knife to the edge using a single hair brush. Care must be taken not to add too much since its relatively high viscosity leads it to being easily dragged over the edge of the knife and on to the sample, at which point it usually becomes necessary to stop and clean the block. Careful sectioning will then lead to sections sliding down the n-propanol into the reservoir. When sufficient sections have been taken, more n-propanol is added to the trough to raise the level and sections are retrieved using a TEM grid. These sections are quickly but gently laid on to the surface of a water-filled petri dish. The interaction between the n-propanol and the water usually leads to the sections being floated off on to the water and flattened out by the action of surface tension. The required sections can then be chosen and removed on a fresh TEM examination grid.

Sectioning without a Trough Liquid

In numerous cases it is found that sectioning with n-propanol is not appropriate. A simple alternative is to section without the use of a trough liquid. The advantage of this is that it is generally easier to judge sections *in situ* and reject those that are unsuitable. Sections are collected on the knife itself and are then carefully positioned on a TEM grid by holding the grid against the knife (away from the knife edge) and manipulating the sections with a single-hair brush. This can be less time consuming than other techniques since it is possible to remove only as many sections as are needed, rather than to continue sectioning until plenty have been removed in the hope that at least some of them will be suitable. The main disadvantage with sectioning dry is that a buildup of static electricity in the chamber, which is a common problem, can lead to sections being difficult to handle and prone either to sticking to the sample or the knife, or flying around the cryo-chamber when attempts are made to move them. Anti-static guns are available but they are expensive and careless use can damage the sensitive electronics of a cryo-unit. There is also no flattening action caused by the surface tension of the water, as in the above case.

It should also be added, at this point, that if the operator is working with unvulcanised materials then this is the technique of choice since interactions between ultra-thin sections and some trough liquids (including water) have been observed. This could lead to distortions in the sections, artefacts and, consequently, misleading information.

Sectioning on to Ice

This is a recently developed technique[8] and is ideal for sectioning materials that have a tendency to curl (*e.g.* NR/BR blends). It is probably the most time consuming technique and can only be used if the cryo-ultramicrotome has an inbuilt defrost unit that automatically raises the temperature to just above ambient. High temperature bake-out units should be avoided for this application unless the bake-out temperature can be set to 35°C–40°C. The RMC CR-21 unit is ideal since it raises the temperature of the apparatus to 35°C. The apparatus is prepared by placing a knife with a trough filled with distilled water in the knife holder. The apparatus is cooled in the usual way and the water in the knife trough will freeze. Sections are cut and arranged on the ice surface so that they are touching without overlapping. After sufficient sections have been taken, the apparatus is warmed until the ice melts. As it turns from ice to slush the sudden increase in surface tension flattens out the sections. (Prior to melting some manipulation of the sections is possible although care must be taken above the T_g not to distort them). Once all the ice has melted, the sections can be retrieved on a TEM grid. This is a time-consuming technique since the apparatus has to be repeatedly warmed and cooled between samples. It does however give good results with difficult specimens.

COMMON PROBLEMS

Curling

Curling during sectioning of vulcanised material usually suggests that the section is too thick. This is solved 'simply' by cutting thinner sections. The most irritating type of curling occurs when seemingly flat sections curl as they are brought up from cryogenic temperatures to room temperature. It seems likely that much of this is due to inbuilt stresses in the material from moulding. While the material is a coherent whole it retains its shape but in some cases, when a section is removed from the bulk cryogenically and allowed to warm to room temperature, the stresses caused the section to curl. This is a particularly common problem in blends containing elastomers with very different T_gs. One good example of this phenomenon is NR/BR blends. As the section is warmed, BR will return to being elastomeric near −110°C (dependent on the type of BR) whereas NR will remain a glass until its temperature is raised above −70°C. The consequence of this is that the BR phase will start to relax out the strains while the NR phase in still a glass. This often leads to the section curling.

There are two possible techniques for overcoming the problem of curling. One of these is the ice-sectioning method described above. The other is to use a triangular block face because this will have a tendency to curl from all three edges resulting in the curl effectively bracing itself and thus leaving a flat region at the centre of the section. This second method is a little less predictable as it depends on the section being the same thickness throughout. However, practice and perseverance can bring some good results.

Knife Marks

These are an inevitable consequence of sectioning an elastomer which contains particulate matter such as zinc oxide, silica, carbon black, *etc.* As the knife cuts through such a specimen and strikes a hard particle the knife may be slightly damaged at that point. From then on, the damage to the knife edge will be translated on to any section and block face, as it passes over that point on the knife, as a long line in the direction of sectioning. Obviously the more filler present in a material the more likely the knife is to be damaged. If a diamond knife is used then the edge will probably not be damaged as quickly, but any damage will be transmitted to any sections taken with that part of the knife until it is resharpened.

Knife marks do, however, have an important use. They can reveal the direction of sectioning which may be important when trying to decide whether the shape of a structure has been influenced by compression (see below). Where the knife marking is severe a computer imaging macro, such as the one written at MRPRA, can be used to improve the visual appearance of an image that has been digitally collected.

Compression

When a section is removed from a block, it often appears to be shorter than the vertical face of the block from which it was removed. This is known as compression. In more severe cases wrinkles appear at right angles to the sectioning direction. These regions should be avoided when taking micrographs. Usually the effect can be reduced by changing the clearance angle and/or the sectioning speed. Bad compression seems to be a result of too high a sectioning speed and/or too steep a clearance angle.

Chatter

Chatter occurs when a high frequency vibration is set up between the specimen block and the knife and leads to regular variations in the thickness of the section. This is observed as parallel lines at right angles to the sectioning direction. As with compression its cause is usually a combination of wrong clearance angle and sectioning speeds.

Inconsistent Sections

Once again in biological circles it is expected that the correct sectioning conditions will lead to a ribbon of ultra-thin sections floating on the water in the trough. It is also expected that once the conditions are correctly set sections will be cut serially, *i.e.* on every cutting stroke. Experience indicates that this is not the case with elastomers. With most technological materials it is unlikely that the operator will be able to stay with any single region of the knife for a prolonged period of time before it becomes blunted. The time taken for this to occur depends largely on the material and on the conditions that the operator is attempting to use. It is quite conceivable that the knife will become blunt after only five or ten cutting cycles and the operator will need to move to the next piece of knife edge. Clearly with the usable knife edge being quite short it is necessary to obtain the correct conditions quickly. With practice and experience a good operator should be able to assess from the type of material what kind of conditions should be set up initially. However, even then it is highly unlikely that serial sections will be cut, or if they are it is unlikely that they will be cut for more than a few sections. Generally speaking the harder the block the steeper the clearance angle that will be required. Sectioning speed is more difficult to assess and seems to depend on too many factors (including operator preference) to be able to give complete guidelines. Generally speaking it is better to start at slow speeds and shallow clearance angles because these will do less damage to the knife edge if they are incorrect.

EXAMPLES

NR/EPDM

This particular blend is an excellent example of how LM can appear to supply all the necessary information. However, once the material has been examined by TEM it becomes clear that there is far more information that can be obtained. The sample in question was prepared at −100°C with a dry glass knife for TEM and using a trough filled with n-propanol for LM. Sectioning was carried out manually and sections were mounted in polybutene for phase contrast LM using a Leitz Ortholux II light microscope or stained in osmium tetroxide for one hour prior to examination using a Phillips EM300 transmission electron microscope. As mentioned above, it has been found that better results are obtained from an instrument running at a higher accelerating voltage and so examinations were carried out at 100kV. Contrast was maximised by using a small objective aperture, in this case 30 µm which is the optimum size for the EM300[9].

Light Microscopy

Figure 4 shows a phase contrast light micrograph that was originally taken at the maximum usable magnification for the technique. The EPDM phase is identified as the lighter phase. The micrograph appears to give a reasonable amount of information regarding blend morphology and a reasonable mean estimate of cross-sectional phase dimensions could be determined. For some applications this limited amount of information would be sufficient.

Transmission Electron Microscopy

Figure 5 shows the same material taken at approximately thirteen times the magnification

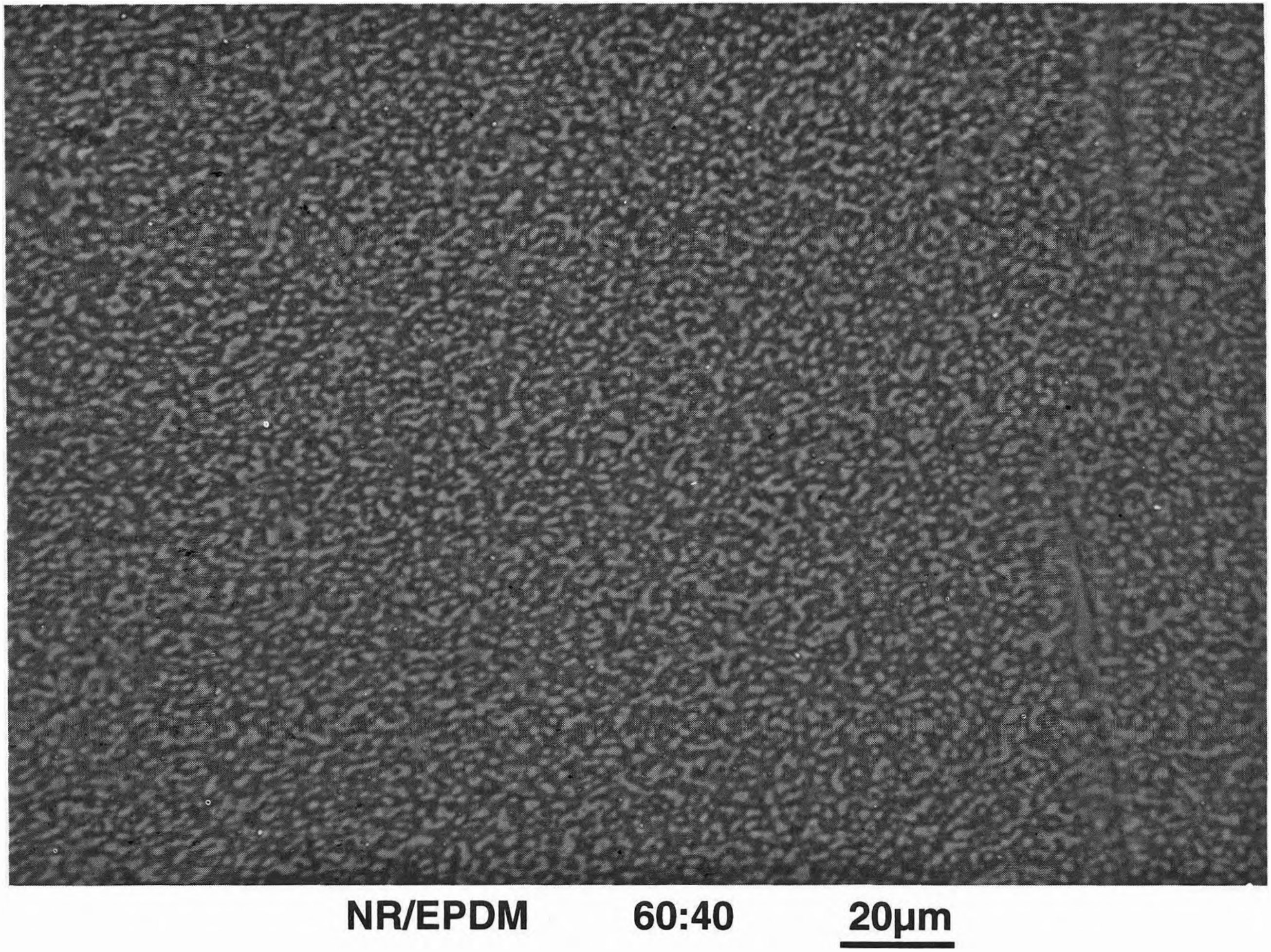


Figure 4. Phase contrast light micrograph of NR/EPDM 60:40.

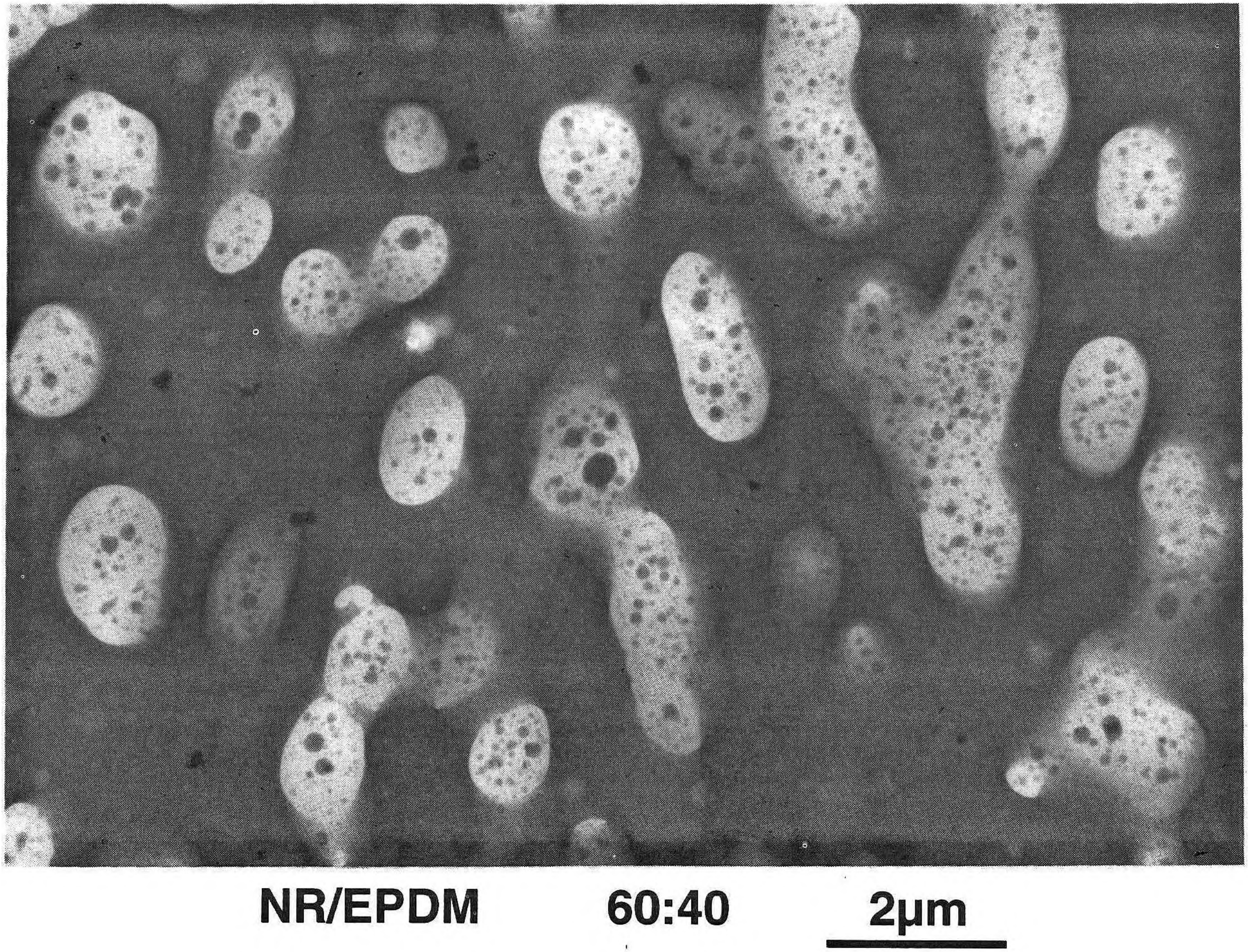


Figure 5. TEM micrograph of NR/EPDM 60:40

used on the light microscope. The EPDM has not been stained by the osmium tetroxide and is the lighter phase. This micrograph is a good illustration of the point made at the beginning of this paper regarding thickness wherein the section is sufficiently thick for the three dimensional nature of the EPDM phase to be observed. Using one's imagination it is possible to mentally extrapolate to produce an image of the NR matrix with the EPDM phase tunnelling through it. A phase microstructure of small micro domains of stained NR sited within the unstained EPDM phase and *vice versa* can also be observed.

NR/CR/NBR

Rubber technologists will be aware of the problems involved in blending NR and NBR. A number of solutions have been successfully devised at MRPRA. One of these is the use of a compatibiliser, in this case chloroprene rubber (CR). The sample was prepared in the same way as the above example. Instrumental conditions were as above.

Light Microscopy

Figure 6 shows a phase contrast light micrograph of this material. A blend morphology can be observed but it is impossible to determine which phase is which since three phases are present but only two can clearly be seen. No conclusions can be drawn regarding the blend morphology or, more importantly, the phase structure of this material.

Transmission Electron Microscopy

Figures 7 and *8* show the same material at magnifications that are thirteen and forty times that of *Figure 6*. The morphology and structure of the blend are far more clear than in the LM micrographs and it can be seen that the CR resides at the interface between the lighter NR phase and the darker NBR phase. The CR can be identified as the darkest of the three phases. Its position is consistent with the expected position of a successful compatibiliser *i.e.* as the 'glue' between two largely incompatible materials. These results therefore confirm that the compatibiliser is working as intended. It would not be possible to obtain this information by light microscopy.

CONCLUSION

The above examples illustrate the increase in the level of information available by TEM by comparison to LM and how important good sectioning technique and an understanding of the processes involved is to successfully obtaining good images. Sectioning elastomers is a complex and time consuming process and despite the advances in preparative equipment there are still reasonable grounds for referring to this as being as much an art as a science. However, any intelligent operator with a reasonable level of dexterity, patience and understanding should be able to develop the necessary skills.

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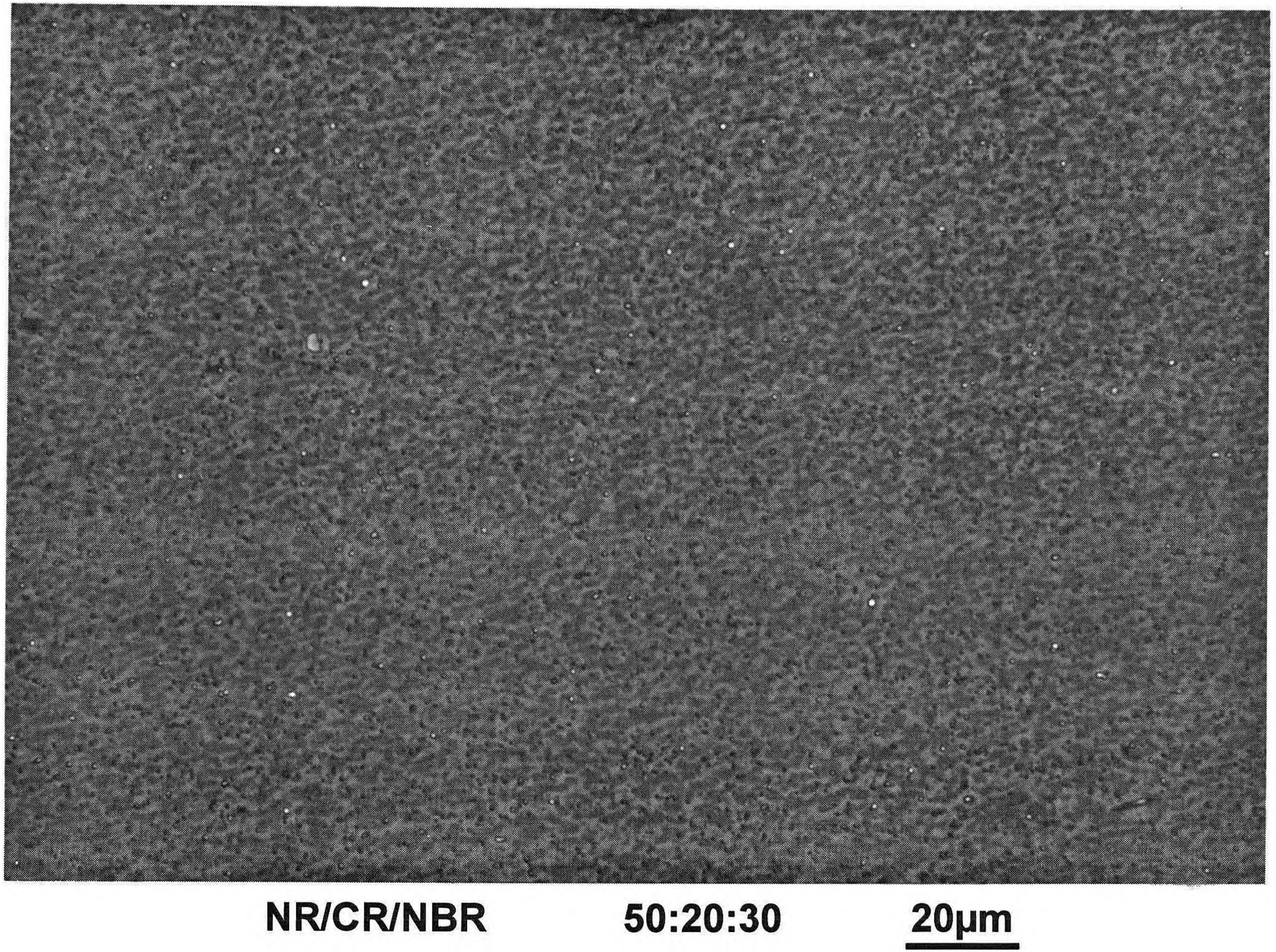


Figure 6. Phase contrast light micrograph of NR/CR/NBR 50:20:30.

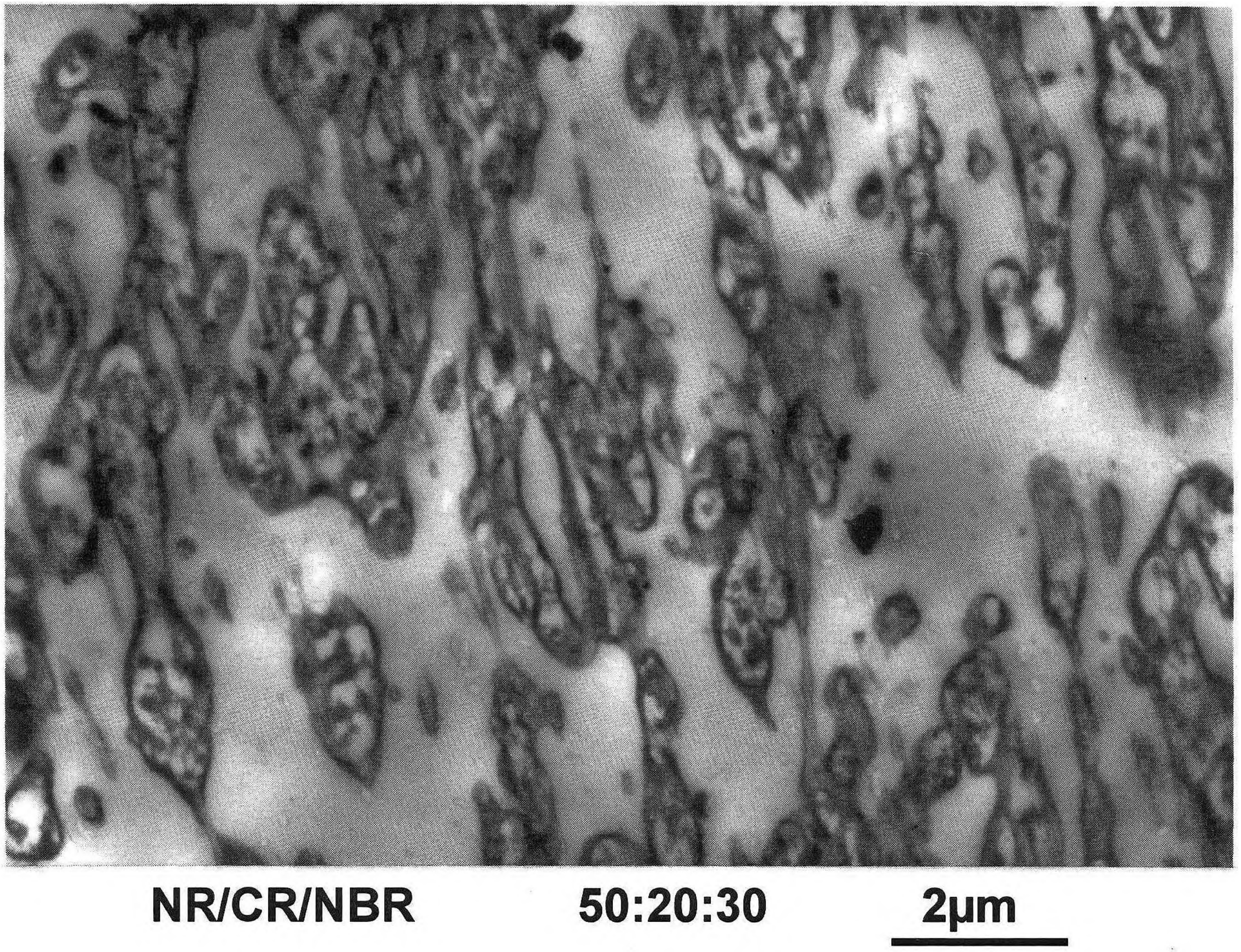
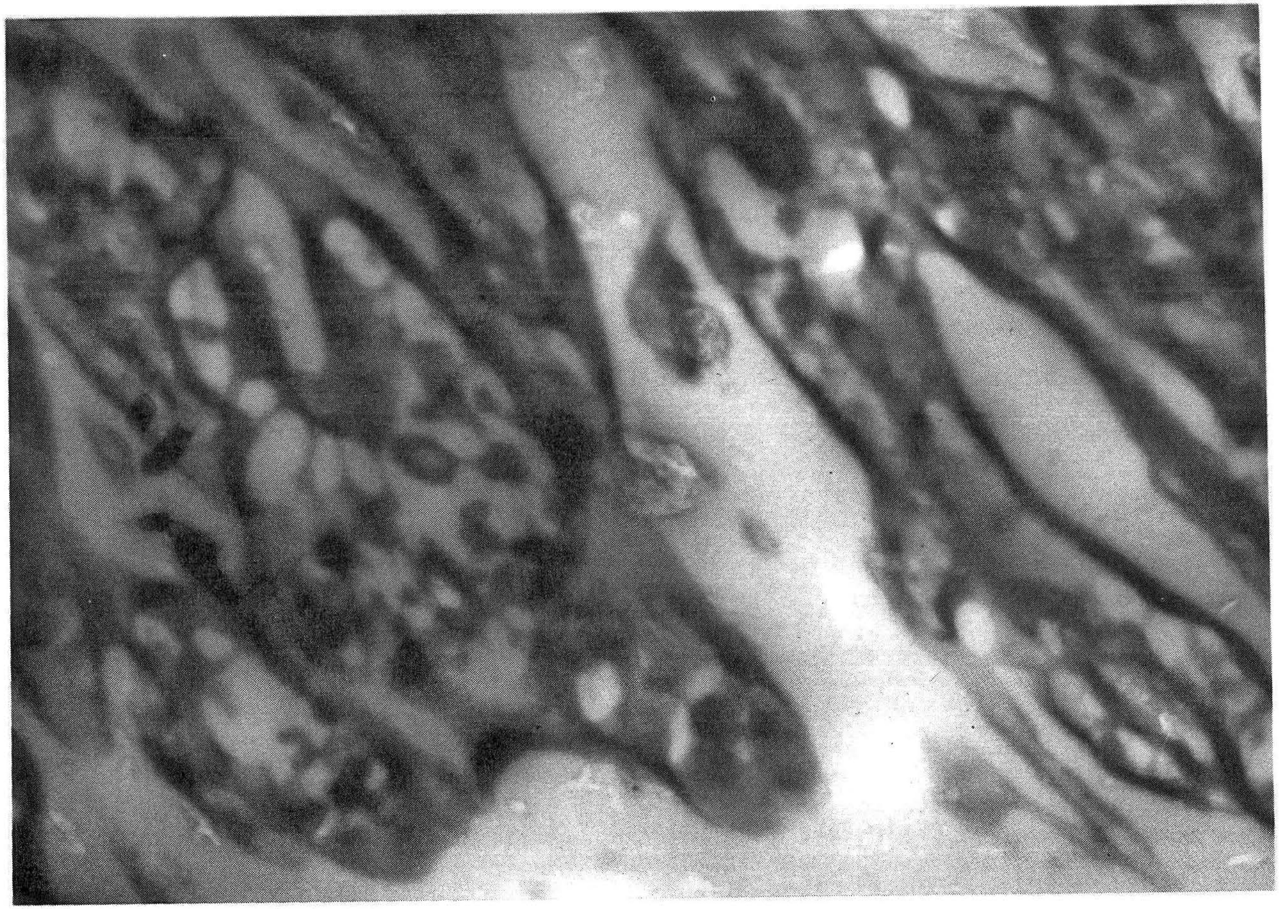


Figure 7. TEM micrograph of NR/CR/NBR 50:20:30.



NR/CR/NBR 50:20:30 0.5μm

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Properties of Rubberised Bitumen from Reclaimed Rubber[†]

AZEMI BIN SAMSURI[*]

This paper discusses the properties of rubberised bitumen prepared by physical blending of bitumen with reclaimed rubber powders obtained from either rejected rubber gloves or scrap tyres. Besides reclaimed rubbers, natural rubber latex and synthetic polymer such as ethylene methyl acrylate were also used to prepare the rubberised bitumen. Properties such as penetration number, softening point, work done to break and tenacity were measured; results showed that the properties improved with the addition of rubber. The softening point, tenacity and energy to break increased progressively while penetration number decreased with increasing rubber content. Rubberised bitumen prepared by using glove crumbs produced overall better properties than that using tyre shavings. The properties of rubberised bituminous mixes were also evaluated and compared with those of ordinary bituminous mixes. The results showed that rubberised bituminous mixes produced higher resistance to permanent deformation and dynamic cracking compared with ordinary bituminous mixes.

Some materials are easy to recycle while others are difficult. Thermoplastic products are easy to recycle and can be moulded into products again simply by the application of adequate heat and pressure. In contrast, most rubber products and thermoset plastics are not easy to recycle and cannot be moulded directly into products again because of the three dimensional crosslink network which prevents flow. Thus, most of the scrap rubber products such as used tyres or rubber discards are presently burnt or buried in designated landfill areas. Both methods are environmentally unfriendly. However, by means of a suitable reclaiming process, reclaimed rubber in the form of fine powders (40 mesh or 420 microns) can be produced from these scrap rubber products (tyres, examination gloves, toy balloons, *etc*.). These fine powders can be used to aid processing such as in extrusion and calendering to reduce extrudate swell and shrinkage and thus provide an overall better dimensional stability. Besides processing aids, reclaim rubbers can be used as extenders to cheapen the compounding costs at the expense of slightly poorer physical properties of the final vulcanisates.

Another important area where fine rubber powders find a wide application is in the construction of flexible road pavement. Currently, an ordinary unmodified bitumen which serves as a binder (a substance used to hold the aggregate structure firmly together or to a substrate) in a wearing course is not adequate to meet the ever increasing traffic/

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axle loads. It is well established that flexible pavements need relaxation time for recovery, a phenomenon known as healing[1]. If the traffic is not heavy (off-peak traffic hours), the small stress cracks within an asphalt pavement will tend to heal or close with time. However, under a very heavy traffic condition, the cracks have little time to heal, thus accelerating pavement failure. Apart from heavy axle loads, climatic factors such as high temperatures and intense heat can also accelerate pavement failure through rapid ageing of bitumen. An aged and degraded bitumen is a hard and brittle material which may form cracks easily due to cyclic stresses associated with traffic loads and expansion and contraction due to thermal changes. The cracks formed will eventually lead to premature failures of road surfaces. If precautions and means of overcoming premature road failures are not carried out, the Public Works Department and Highway Authority will continue to pay a very high cost for road maintenance and repairs. Furthermore, driving on defective road surfaces is both uncomfortable and unsafe.

Increasing demands on road surfacings posed by higher traffic density, heavier loads and faster speeds emphasise the need for binders with improved performance. It is in this area that rubberised binders have a role to play. Quite small quantities of rubber improve the cohesive strength of bitumen; the latter becomes tougher and more tenacious than unmodified bitumen[2]. Rubber makes the bitumen less brittle and increases the range of temperatures over which it remains serviceable; the flexibility which rubber imparts allows it to hold more securely to aggregate against displacement forces[2]. The addition of rubber particulate increases the viscosity of the bitumen cement and, thereby, improves the bitumen's film thickness on the aggregates, allowing the hot mix to hold more binder content and at the same time reduce the absorbency of aggregates[1]. This, in turn, can help optimise the binder content to the advantage of the pavement design-engineer.

The studies on the incorporation of rubber and synthetic polymer into bitumen to improve the properties of the binder for road pavement date back to the 1930s[3]. The usage of polymer-modified bitumen to improve the performance of pavement system is now widely adopted in Europe, United States of America and Australia. However, the knowledge and technology regarding the use of natural or synthetic rubber as an additive to bituminous binder are still relatively new in the South East Asian regions. In view of this scenario, colloborative work was undertaken by the Rubber Research Institute of Malaysia (RRIM) and Public Works Department Research Institute (IKRAM) with a view to provide knowledge and information to the local road engineers on the use of natural rubber additive to modify the bituminous binder as a means or an alternative to overcome the problems of premature failure of road pavement.

This paper discusses some of the laboratory studies and limited service trial of rubberised-bituminous mix to explore the usage of fine rubber powders as bitumen modifier in making road pavements. The fine rubber powders were produced from natural rubber examination glove rejects and tyre shavings, respectively. Apart from reclaimed rubber, natural rubber latex concentrate and polyethylene-co-methyl acrylate (EMA) were also used to modify the bitumen. The performance of rubberised-bitumen based on fine rubber powders obtained from scrap rubber goods was compared with that of rubberised bitumen based on natural rubber latices and also with that of unmodified bitumen.

EXPERIMENTAL

Materials

Four different types of rubber were used *viz.* natural rubber (NR) latex concentrate, prevulcanised NR latex, fine rubber powders reclaimed from rejected examination gloves (particle size of 0.5 mm) and tyre shavings (particle size of 0.5 mm). Commercial grade EMA was also used to modify the bitumen (details of technical specifications were not disclosed). Bitumen of 80/100 penetration number was used throughout unless stated otherwise.

Mixing

Mixing was carried out on a laboratory scale using a Hobart mixer at a temperature of 160°C for about 1.5 h. First, the solidified bitumen was heated until it melted and in liquid form. On reaching the required temperature, the bitumen was blended with rubber at rotor speed of 80 r.p.m. The quantity of rubber was varied from 2 parts per hundred of bitumen (p.p.h.b.) to 10 p.p.h.b. Large scale mixing was carried out in a blending tank of 10 metric ton capacity; only the fine rubber powders reclaimed from reject examination gloves were used in the large scale mixes.

Road Trial

A rubberised-bitumen road trial using fine rubber powders from reject gloves and NR latex concentrate was carried out on the N1 Rembau road, Negeri Sembilan. The amount of rubber used was five percent of the binder content. The quantity of rubberised bituminous binder used was five percent by weight of the total mix aggregates. There were two methods of producing the mix. In the first case, the rubberised bitumen was first prepared by preblending fine rubber powders with bitumen in a 10 metric ton capacity tank mixer (as decribed above). Then, the preblended rubberised bitumen was mixed with the aggregates of different sizes at different proportions in accord with gradation SHRP mix design. Mixing of rubberised bituminous binder with the aggregates was carried out in a pugmill by using the batch process. In the second case, instead of preblending the rubber with bitumen, the rubber was added directly to bitumen and aggregates where they were mixed in the same pugmill. Samples were taken from the bituminous mix to prepare the cylindrical test-pieces by using a gyratory compactor which simulated field compaction. A pressure of 240 kPa was applied for 200 revolutions at 135°C. In this investigation, sample designated by SX 20 denoted a dense bituminous mix where unmodified bitumen was used as the binder. SX 20 + NR latex denoted a dense mix prepared by the second method where rubberised bitumen (based on NR latex) was used as the binder. SX 20 + glove crumbs denoted a dense mix prepared by the second method where rubberised bitumen (based on glove crumbs) was used as the binder. SX 20 + preblended glove crumbs denoted a dense mix prepared by the first method where rubberised bitumen was used as the binder based on preblended glove crumbs.

Physical Tests

Penetration number. The penetration number was determined according to *BS 2000: Part 49*, 1983. It is a measure of consistency of a bituminous material expressed as the distance in tenths of a millimeter that a standard needle vertically penetrates a sample of the material under known conditions of loading, time and temperature. In this test, the sample was melted and filled in a penetration cup (diameter 55 mm, internal depth 35 mm). Later, the sample was cooled and placed together with a transfer dish in water bath at 25 ± 0.1°C for 1.5 h. The penetration was measured with a penetrometer by means of which a standard needle was applied to the sample under a fixed known load (100 g). The distance (depth) of penetration was measured after 5 seconds of loading.

Softening point. The softening point was determined using ring and ball apparatus according to *BS 2000: Part 58*, 1983 specifications. In this test, a steel ball of specific mass was placed upon a disk of bitumen contained within a metal ring of specified dimensions. The assembled apparatus was placed in a bath of liquid and the softening point was taken at which the bitumen surrounding the steel ball just touched the base of the apparatus.

Energy at break and tenacity. Rubberised-bitumen sample was melted by heating at a temperature of 120°C. The molten sample was poured into three mould brickets until it excessively filled the mould cavity and allowed to cool at 23°C. Once the sample solidified, the excessive material was removed with a hot knife. The sample was conditioned at 23°C for 24 h before testing. The stress-strain measurements on rubberised bitumen samples in a moulded bricket were carried out by using an Instron tensile machine at a temperature of 23°C. The crosshead speed was 500 mm per min. The typical load-deformation curve is as shown in *Figure 1* and *Figure 2* for unmodified bitumen and rubberised bitumen, respectively. The energy to break was determined by integrating the area under the load-deformation curve OYE. In the case of unmodified bitumen, it is difficult to locate the breaking point since the bitumen just continued to flow as shown in *Figure 1*. To overcome the problem, the breaking point was referred to that of the breaking point of the rubberised bitumen.

The tenacity was determined by integrating the shaded area as shown in *Figures 1* and *2*. A straight line was extrapolated from the straight portion of the curve to meet the x-axis. The tenacity is defined by the area bounded by the straight line and the curve in the flow region (unmodified bitumen) or the curve in the 'rubbery' region (rubberised bitumen) as shown by the shaded portion in the two figures.

Marshall Test. The test was originally developed by Bruce Marshall and is described in *BS 598: Part 3*, 1985. This test is used to measure the resistance to plastic flow of cylindrical specimens of bituminous paving mixture loaded on the lateral surface by means of the Marshall apparatus. In this test, a cylindrical specimen (diameter 100 mm; length 60 mm) was compressed diametrically at a constant rate (50.8 mm per minute), at 60°C until failure. The maximum load developed during the test, known as Marshall stability, and the deformation at the maximum load, known as the Marshall flow were measured. Marshall quotient was determined by dividing Marshall stability by Marshall flow.

Indirect tensile modulus and fatigue tests. Both tests were carried out using the materials testing apparatus for asphalt (MATTA) machine as shown in *Figure 3*. The machine consists of a loading frame which has a heavy,

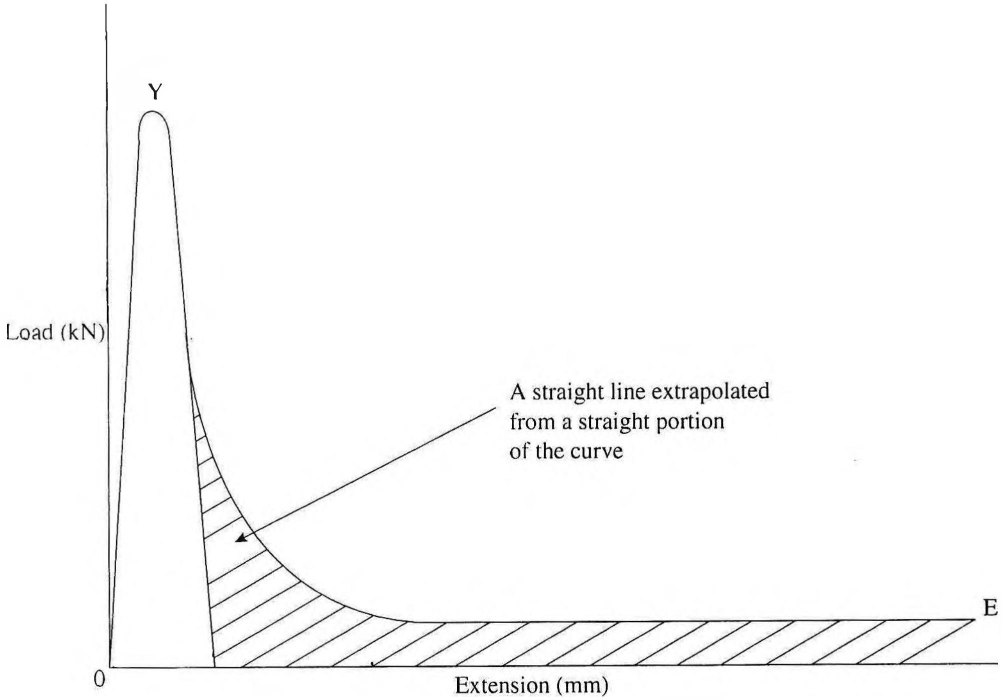


Figure 1. Load-deformation curve of unmodified bitumen.

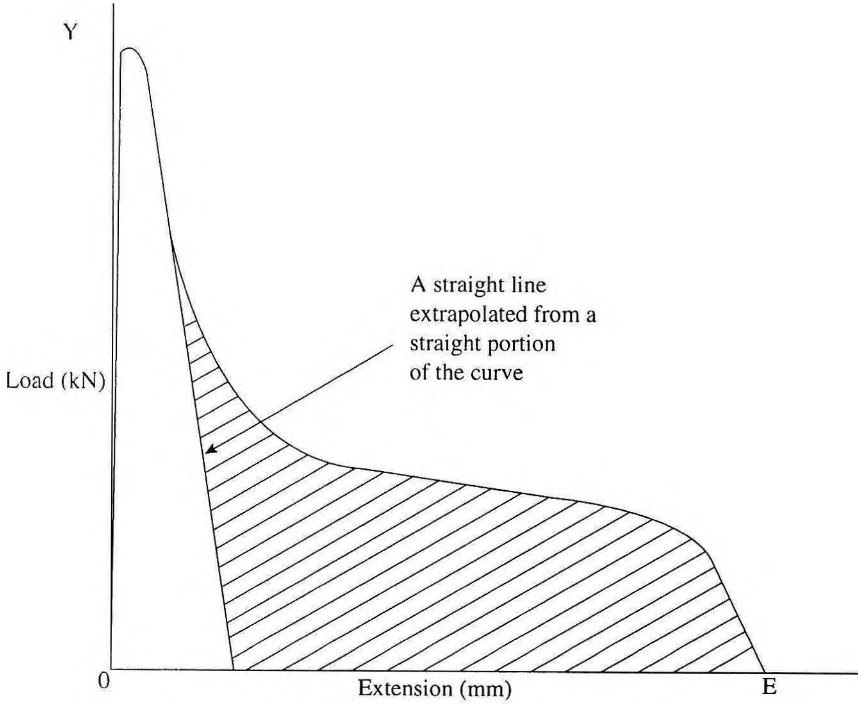


Figure 2. Load deformation curve of rubberised bitumen.

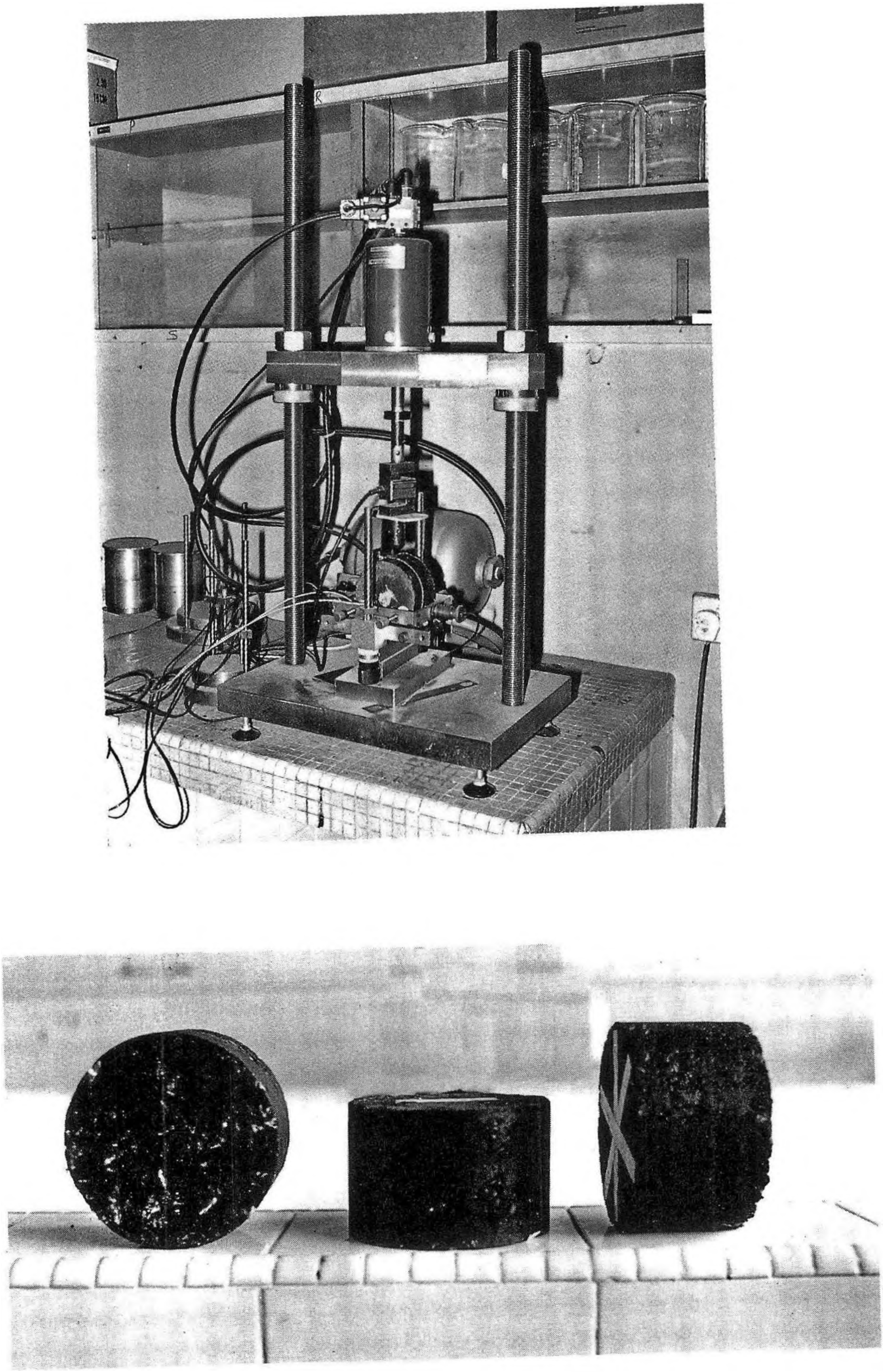


Figure 3. Photographs showing MATTA machine and test-specimen.

flat, baseplate, supported on four levelling screws. The frame is of heavy construction to limit deflection and vibrations which could influence the accuracy of measurements during dynamic repeated loading tests. Loading forces are applied through the shaft of a pneumatic actuator mounted in the centre of the crosshead. A strain gauged force transducer, mounted in line with the loading shaft, measures the force applied to the specimen. The cylindrical specimen (as decribed above) is mounted in jigs that enable either indirect tensile or axial compressive loading.

The indirect tensile fatigue test provides facilities for repeated loading of the sample in indirect tensile mode for a maximum of one million pulses. Total and permanent strain together with modulus are continuously plotted against the number of pulses on logarithmic scales.

RESULTS AND DICSUSSIONS

Properties of Rubberised-bitumen

Effect of type and quantity of rubber on penetration number. The effect of type and quantity of rubber on penetration number is shown in *Figure 4* where penetration number is plotted against rubber content, based on the data shown in *Table 1*. Unmodified bitumen is a soft and viscous material as reflected by its high penetration number, *i.e.* 85.8. The penetration number was affected both by the type and quantity of rubber. All types of rubber showed a similar trend, *i.e.* the penetration number decreased progressively with increasing amount of rubber indicating the material became more viscous and harder. Blending rubber with bitumen results in a binder with improved hardness similar to harder grade bitumen. At more than 5 p.p.h.b. EMA was the most effective in decreasing the penetration as reflected by the lower penetration number compared with rubbers as the modifier. Among the rubbers, fine rubber powders reclaimed from reject gloves and prevulcanised NR latex were very effective in decreasing the penetration. The result also showed that it required twice as much tyre shavings to get the same penetration number produced by the fine rubber powders from reject gloves. This might be due to higher rubber hydrocarbon content in reject gloves than in tyre shavings since the latter contained 40 to 50 parts p.h.r. of carbon black.

Softening point. Figure 5 shows the plot of softening point *versus* rubber content, based on the data shown in *Table 2*. Unmodified bitumen has a low softening point of about 44°C. Incorporation of rubber into bitumen generally increases the softening point of the modified binder which has an advantage of the possibility of reducing the tendency to 'bleed' in hot weather which is a common problem for pavements in warmer climates[4]. The plot in *Figure 5* shows a similar trend for each type of rubber. The softening point increased progressively with increasing rubber content. EMA modified bitumen produced the highest softening point followed by NR latex and fine rubber powders (reject gloves), while tyre shavings modified bitumen produced the lowest softening point among the rubbers. The result again showed that it required at least twice as much or more tyre shavings to obtain the softening point produced from fine rubber powders of reject gloves which might indicate that, it is the rubber hydrocarbon content which predominantly modifies the bitumen.

Work done and energy at break. Figure 6 shows the load-deformation characteristics of rubberised bitumen as well as the unmodified

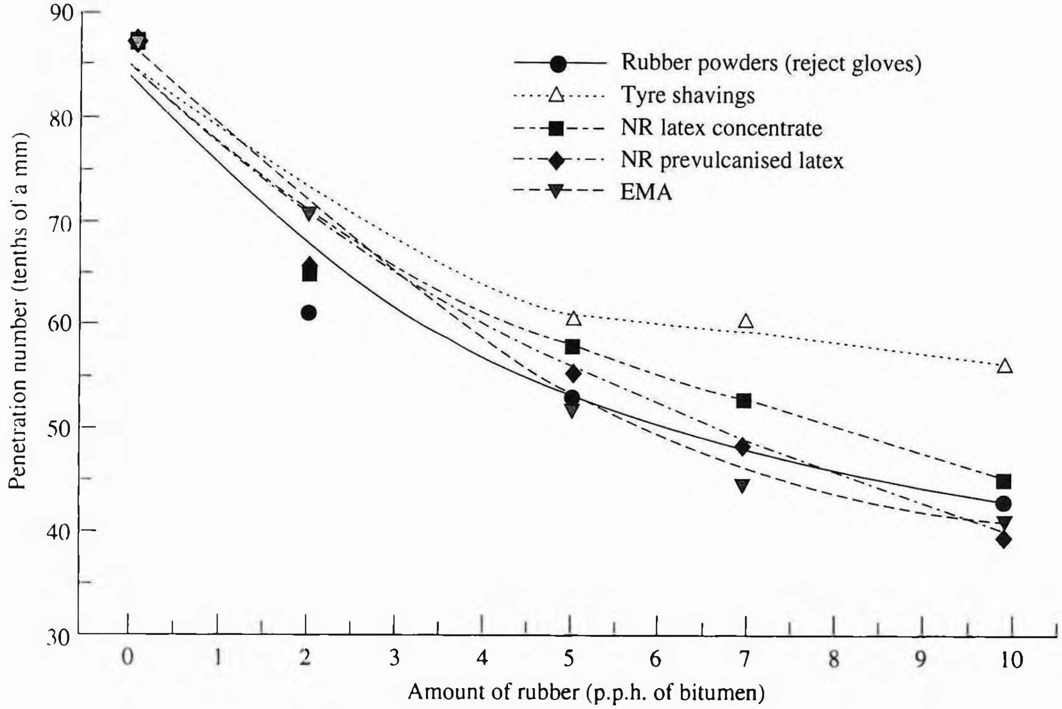


Figure 4. Penetration number vs *amount of rubber.*

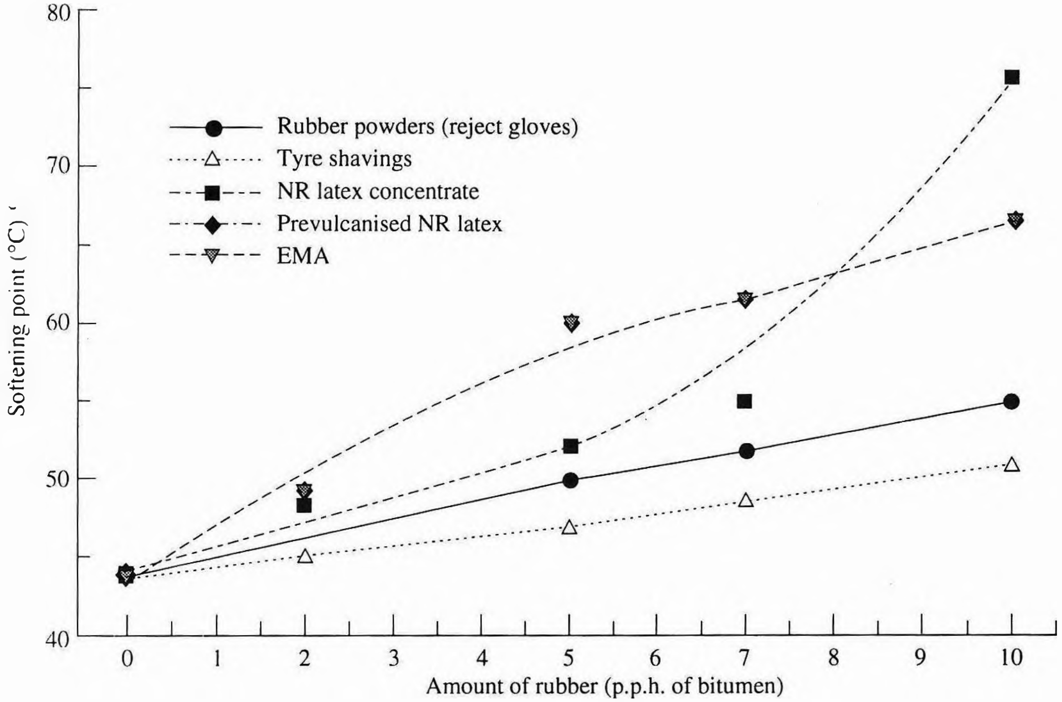
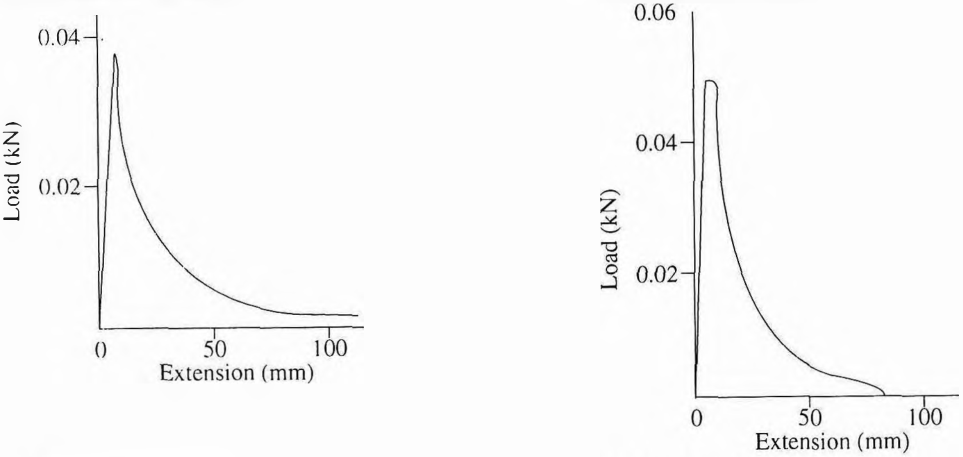


Figure 5. Softening point vs *amount of rubber.*

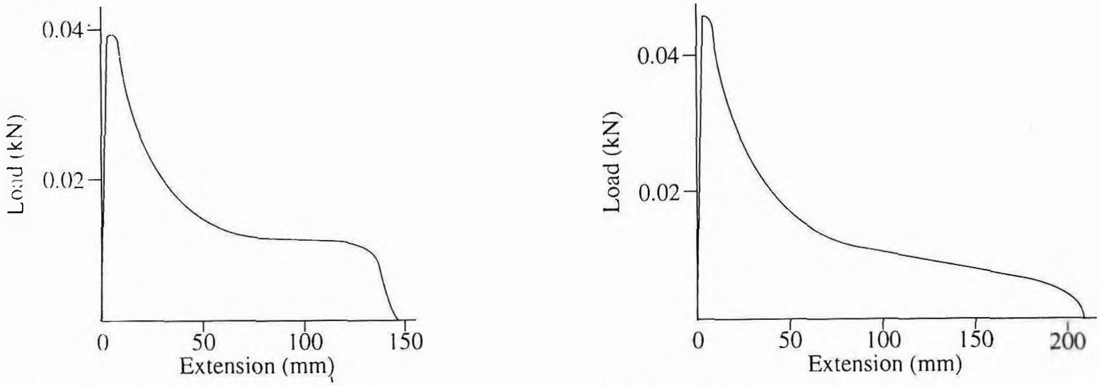
(a) Unmodified bitumen

(b) 5% tyre shavings



(c) 5% glove crumbs

(d) 5% NR latex concentrate



(e) 5% prevulcanised NR latex

(f) 5% EMA 503

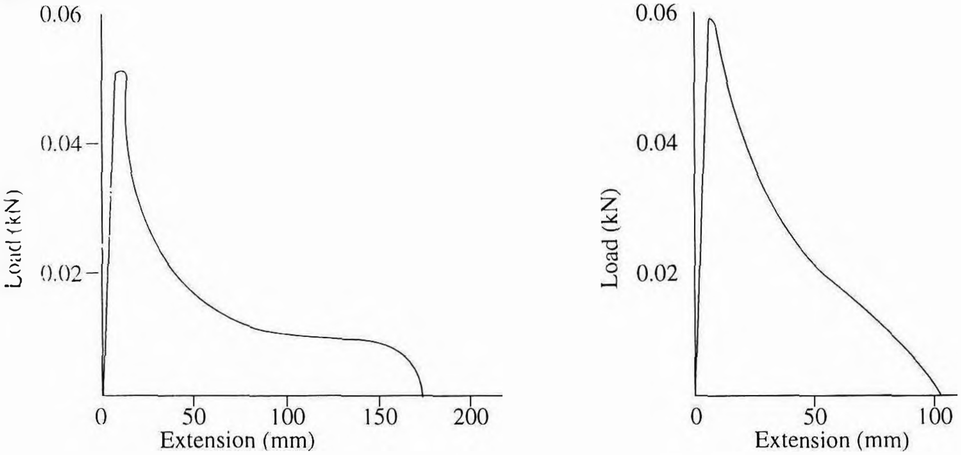


Figure 6. Load displacement characteristics of different types of rubberised bitumen reproduced from the chart recorded during the experiment.

TABLE 1. PENETRATION NUMBER (TENTH OF A MM) OF RUBBERISED-BITUMEN

Amount of rubber (p.p.h. of bitumen)	2	5	7	10
Rubber powders (reject gloves)	60.4	52.0	47.6	42.6
Tyre shavings	64.4	60.2	59.4	54.6
NR latex concentrate	64.4	57.0	51.8	44.4
Prevulcanised NR latex	64.8	54.8	47.6	39.4
EMA	69.2	51.2	44.2	41.0

Penetration number of unmodified bitumen = 85.8 (tenths of a m.m.)

TABLE 2. SOFTENING POINT (°C) OF RUBBERISED-BITUMEN

Amount of rubber (p.p.h. of bitumen)	2	5	7	10
Rubber powders (reject gloves)	47	50	52	55
Tyre shavings	45	47	48	50
NR latex concentrate	47	52	56	75
Prevulcanised NR latex	48	60	62	66
EMA	48	60	62	66

Softening point of unmodified bitumen = 44°C

bitumen and EMA-modified bitumen. The work done or energy to break was determined from the area under the load-deformation curve. The load-deformation curve was affected by the type of rubber (the amount used in each case was 5 p.p.h.b.). Unmodified bitumen produced a low maximum load after which the load decreased continuously producing a curve that looks like a rectangular hyperbola which asymptotes with the x-axis as the unmodified bitumen just continue its flow during deformation. In the case of rubber-modified bitumen, the maximum load increased markedly after which the load decreased progressively to a value about one third of its maximum load (in the cases of fine rubber powders from reject gloves and NR latices) before producing a 'rubbery' plateau region as a consequence of some degree of resistance to deformation attributed to the elastic behaviour of the rubber. Bitumen modified with tyre shavings and EMA did not produce broad plateau region; perhaps these materials are less rubbery since tyre shavings are filled with high concentration of black fillers and EMA is a plastic. Nevertheless, all modified bitumen binders showed a definite breaking point unlike unmodified bitumen which continued to flow like a thin thread even after the crosshead had reached its maximum permitted distance travelled.

The plot of work done or energy to break *versus* quantity of rubber is as shown in *Figure 7*. The unmodified bitumen when compared at the same breaking deformation as that of rubberised bitumen has the lowest energy, about 0.46 J, indicating that the material is weak and easily deformable. Addition of rubber into bitumen improved the energy to deformation significantly as reflected by the increase in the work done (energy) to break. Among the rubbers used, NR latex concentrate is the most effective in enhancing the energy to deformation, followed next by fine rubber powders from reject gloves. Addition of 5 p.p.h.b. of fine rubber powders from reject gloves, increased the energy to deformation by a factor of about four compared to that of unmodified bitumen. Thus, the results show that rubberised bitumen is very effective in enhancing the energy to deformation.

Effect of the rate of deformation on energy to break. The viscoelastic behaviour of rubber-modified bitumen was investigated by performing tensile stress-strain measurements at different rates of deformation at 23°C. This study is useful since in practice, the road pavements are subjected to different rates of cyclic loading as a consequence of different masses and accelerations of traffic axle loading. In this study, rubberised bitumen containing 5 p.p.h.b. of fine rubber powders reclaimed from reject gloves was subjected to different rates of deformation by pulling a sample in a mould bricket at different crosshead speeds ranging from 10 to 500 mm per minute using an Instron tensile machine. A similar exercise was also carried out for the unmodified bitumen. The results are shown in *Figure 8* where the energy to break is plotted against crosshead speed. Generally, the energy to break increased with increasing rate of deformation. The energy to break for both unmodified bitumen and rubberised bitumen increased by a factor of about four by increasing the crosshead speed from 10 mm per minute to 500 mm per minute. This is clearly a manifestation of the viscoelastic effects of the material. At any rate of deformation, the energy to break of rubberised bitumen is always higher than that of unmodified bitumen by a factor of about two at the lowest rate and by a factor of about four at the highest rate of deformation.

Tenacity. High tenacity is required for bitumen to be used in drainage mixes for good and long service performance^5. A plot of tenacity *versus* amount of rubber is as shown in *Figure 9*. Unmodified bitumen produced a low tenacity value, 0.17 J. Addition of rubber into bitumen improved the tenacity of the modified binder. NR latex concentrate appears to be the most effective in enhancing the tenacity of the modified binder, followed by fine rubber powders from reject gloves.

Properties of Rubberised-bitumen Mix

Marshall test. The Marshall test can be used to predict the resistance to permanent deformation of bituminous mixes on the basis of empirical relationships. The Marshall quotient has been found to correlate with permanent deformation better than either the individual value of stability or flow. The results of the Marshall test are shown in *Table 3*. The addition of rubber give rise to an increase in the Marshall stability and quotient. The increase varies with the form of rubber used and the method of incorporating the rubber into

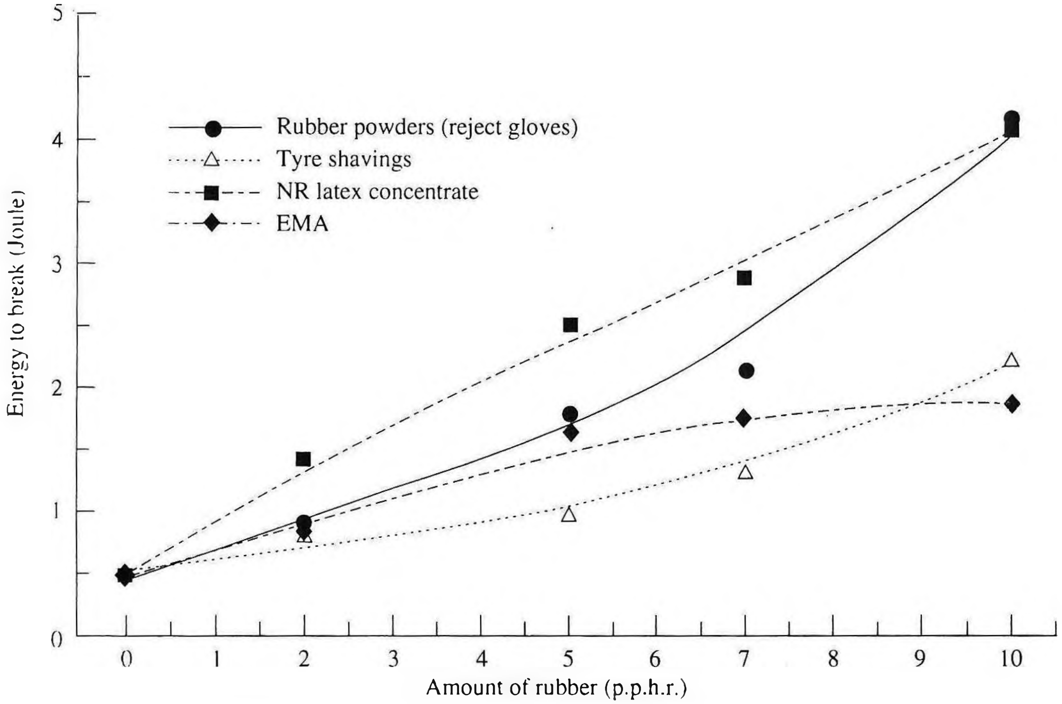


Figure 7. Energy to break vs *amount of rubber (crosshead speed 500mm/min at 23°C).*

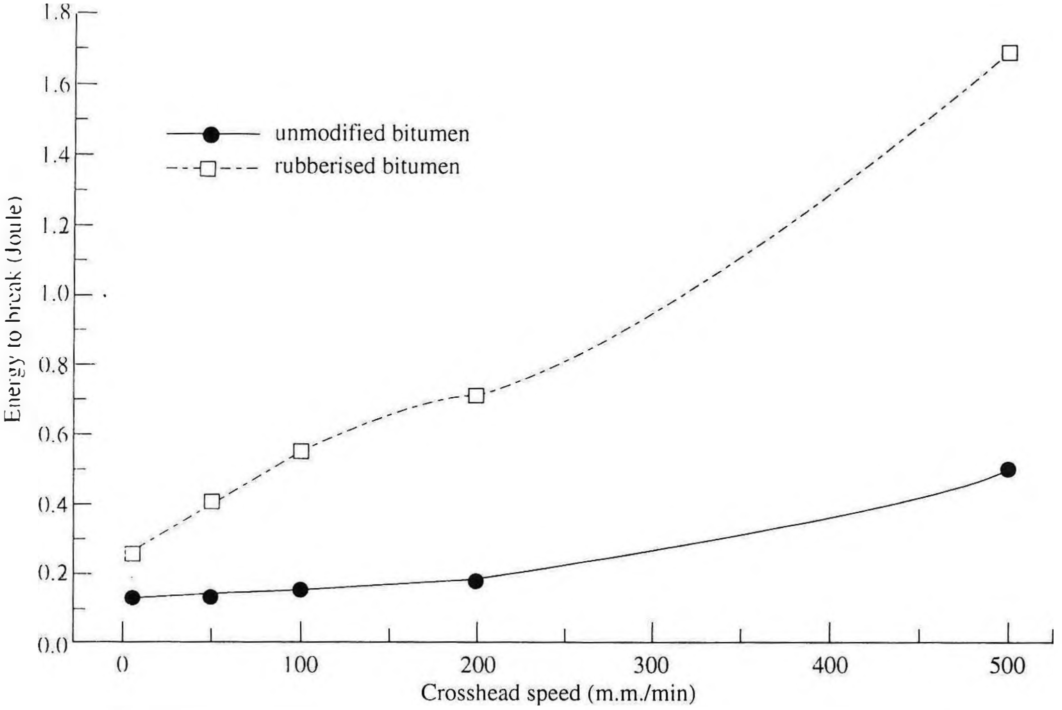


Figure 8. Energy to break vs *crosshead speed (effect of rate on energy to break at 23°C).*

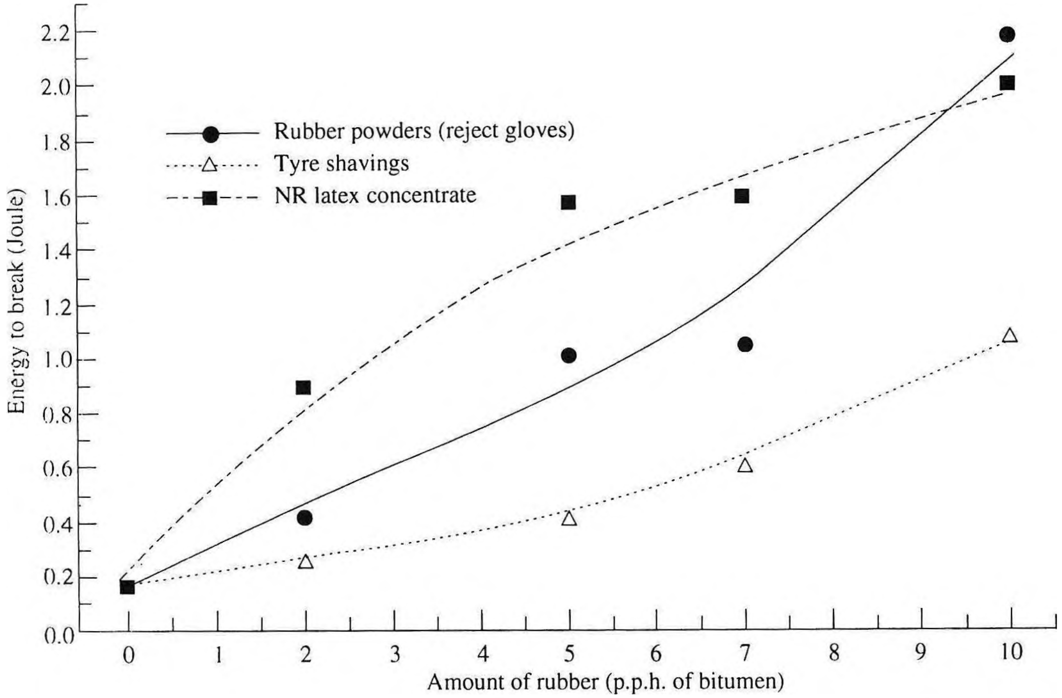


Figure 9. Tenacity versus *amount of rubber (crosshead speed 500 mm/min at 23°*C).

bitumen. Mixes produced using bitumen pre-blended with fine rubber powders clearly show the greatest improvement, causing an increase in the Marshall stability by more than two folds and in the Marshall quotient by nearly three folds compared to the normal unmodified bituminous mix (SX 20). Direct mixing of rubber with bitumen and aggregates (*i.e.* mixing *in situ*) in the pug mill produced rubberised bituminous mixes with an increase in Marshall stability by 1.4 times and in the Marshall quotient by 1.7 times higher than unmodified bituminous mix. Thus, pre-blending of bitumen with rubber is a necessary step in order to produce an efficient rubberised bitumen binder probably due to adequate and efficient rubber dispersions in the bitumen phase.

Indirect tensile modulus test. The indirect tensile modulus test is a quick, non-destructive method of measuring the stiffness of bituminous mixes under dynamic loading conditions to which the material is subjected in practice. In general, the higher the stiffness, the better is its resistance to permanent deformation. *Table 4* shows the results of indirect tensile modulus test. The effectiveness of the pre-blended fine rubber powders is again evident in the indirect tensile modulus test. The elastic modulus of the samples produced using the pre-blended rubberised bitumen is approximately three times greater than that of the unmodified bitumen samples which is in accord with the Marshall quotient results. Thus, the presence of binder modified by preblending with fine rubber powders improved the

TABLE 3. RESULTS OF MARSHALL TEST

Mix	Marshall stability (kN)	Marshall flow (mm)	Marshall quotient (kN/mm)
SX 20	7.62	5.77	1.32
SX 20 + NR latex	13.42	5.44	2.47
SX 20 + glove crumbs	10.90	4.76	2.29
SX 20 + pre-blended	16.12	4.89	3.30

TABLE 4. RESULTS OF INDIRECT TENSILE MODULUS TEST

Mix	Elastic modulus (MPa)
SX 20	2 379
SX 20 + NR latex	2 932
SX 20 + glove crumbs	3 175
SX 20 + Pre-blended glove crumbs	6 579

resistance to permanent deformation by nearly three times than that of unmodified bituminous mixes.

The high elastic modulus of rubberised bituminous mixes has another advantage, *i.e.*, it improves the resistance to rutting. Rutting is accumulation of permanent strain which occurs in two phases. Initially, it is that of densification of the mix, followed by plastic flow of the mix. Rutting usually occurs while climbing lanes and also near roundabouts where vehicles travel at a very low speed. Rutting of road surfaces is hazardous as it disturbs the control of vehicle passing over it. It was reported by the Public Works Department that the addition of natural rubber as an additive to bituminous binder improves the resistance to rutting in the wheel tracking test by a factor of about six compared to unmodified bituminous mix[6].

Indirect tensile fatigue test. The results of indirect tensile fatigue test are shown in *Table 5.* When subjected to repeated indirect tensile loading of 2000 Newton, all samples that had been modified with rubber show higher resistance to fatigue stress. The preblended fine rubber powder samples again performed the best in this fatigue test. While it required more than twice and four times the number of load pulses to reach a permanent strain of 10^{-3} for the directly added latex and rubber powder samples, respectively in comparison to the unmodified bitumen samples (200 pulses); no

TABLE 5. RESULTS OF INDIRECT TENSILE FATIGUE TEST

Mix	Appropriate number of load pulses to reach permanent strain of 10^{-3}
SX 20	200
SX 20 + NR latex	550
SX 20 + glove crumbs	950
SX 20 + Pre-blended glove crumbs	*

*Test terminated after 1000 pulses. No permanent strain was recorded by then.

permanent strain was induced in the pre-blended rubber powder samples after 1000 pulses. Thus the results again shown the better performance of rubberised bituminous mixes than unmodified bituminous mixes in terms of resistance to permanent deformation and fatigue cracking.

Cost effectiveness. Table 6 shows an estimated cost to produce a road pavement section having a length of 200 m, width of 7 m and thickness of 0.05 m base on different types of modified binders and that of unmodified bitumen. The amount of pre-mix required is about 168 metric tons based on 5% binder (by weight). The cost shown in the table includes the labour, processing, construction, raw materials, and overheads, but excludes transportation cost.

The total cost to make that section of road pavement using unmodified bitumen is about RM14 280 (RM = Malaysian dollar) or about US$5712. If fine rubber powders reclaimed from reject gloves are used to modify the bitumen, the total cost is about RM16 113 (US$6445.20). An increase in cost of about 13%. The cost of rubber powders reclaimed from reject gloves is about RM1.80 per kg.

If NR latex concentrate is used to modify the bitumen, the total cost to make a similar road pavement is about RM19 645 (US$7858). An increase in cost of about 35% compared to unmodified bitumen.

IF EMA is used to modify the bitumen, the total cost is about RM25 045 (US$10 018). An increase in cost of about 75%.

Among the three modifiers, rubber powders reclaimed from reject gloves is the most cost effective. The Marshall quotient of preblended rubberised bitumen is about three times higher than that of unmodified bitumen indicating that the life span of rubberised bitumen pavement is about three times longer than unmodified bitumen pavement[6]. Thus, the effective cost of rubberised bitumen pavement is actually RM5371 (*i.e.*, RM16 113 ÷ 3), about 2.7 times cheaper in terms of maintenance cost.

DISCUSSIONS

The results discussed above favour the use of rubberised bitumen as an alternative binder instead of unmodified bitumen because of the improvement in the physical properties of both

TABLE 6. ESTIMATED COSTS TO PRODUCE ROAD PAVEMENT SECTION (200 m × 7 m × 0.05 m) USING DIFFERENT TYPES OF MODIFIED BITUMINOUS BINDER

Type of binder	Cost RM	 $US
Unmodified bitumen	14 280	5 712
Rubber powders (reject gloves)	16 113	6 445
NR latex concentrate	19 645	7 858
EMA	25 045	10 018

RM = Malaysian ringgit (dollar) RM2.50 = US$1.00

the modified binder and that of the rubberised bituminous mixes. Rubberised bitumen has a better temperature susceptibility and less susceptible to temperature changes compared to unmodified bitumen. Rubberised bitumen also has a better resistance to deformation and higher tenacity compared to unmodified bitumen. Rubberised bituminous mixes are predicted to last at least three times longer than unmodified bituminous mix based on the Marshall quotient and elastic modulus results. The question now is the type of rubber most appropriate to use to modify the bitumen. Among the rubbers used, it appears that both NR latex concentrate and pre-vulcanised NR latex are the most effective in enhancing both the physical properties of the modified binder and rubberised bituminous mixes. However, other relevant factors such as the environment, ease of processing and cost should be considered before deciding the suitability of the rubber.

On a laboratory scale it is easy to blend NR latices with bitumen where the experiment can be easily controlled and monitored. However, on a large factory scale, it is not very easy to control and handle the problems associated with foaming, steaming, liberation of ammonia gas and furthermore, if improper stabilisers are used, gellation of the latex may occur due to heat at high temperatures and mechanical agitation. If gellation of latex occurs pre-blending with bitumen is not possible. The liberation of ammonia gas mentioned earlier will also cause pollution to the environment and needs proper effluent treatment to combat the problem.

On the other hand, fine rubber powders reclaimed from reject gloves offer a few advantages if they are used as modifier. The advantages with regards to the environmental factors are that it helps to minimise the problems associated with disposing reject gloves and it reduces the problems of pollution associated with burning, burying or disposing discarded gloves into rivers or seas.

The advantages concerning processing and cost are that it is easy to blend rubber powders with bitumen without any problems provided that care is taken to choose the correct temperature and speed of mixer; and the cost of rubber powders obtained from reject gloves is cheap, about three times cheaper than NR latex concentrate.

In view of the greater advantages of rubber powders than NR latices in terms of environment friendly, ease of processing and handling and cost of material, rubber powders reclaimed from reject gloves is a very suitable material to modify the bitumen to improve the physical and mechanical properties of the binder and the rubberised bituminous mixes.

CONCLUSION

Addition of rubber into bitumen improves both the physical and mechanical properties of the binder and rubberised bituminous mixes, in particular, in terms of temperature susceptibility and resistance to permanent deformation. Thus, this will lead to an improvement in the service life of the road pavement and hence the overall maintenance cost will be very low.

ACKNOWLEDGEMENT

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Stress Relaxation Behaviour of Natural Rubber Vulcanisates Containing Non-rubber Constituents

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Natural rubber (NR), which is derived from latex of Hevea brasiliensis *tree, contains about 3%–5% of non-rubber constituents. The presence of these non-rubber constituents, which are mostly proteinaceous materials, affect the sensitivity of rubber to water, thereby affecting properties such as elastic modulus and stress-relaxation.*

Studies using vulcanised unfilled NR showed that changes in relative humidity of the sample gave variations in elastic modulus and stress-relaxation of vulcanisates. The variability was due to the presence of certain types of amino-acid; their presence increases the elastic modulus and reduces the rate of relaxation of NR vulcanisates. These were due to the formation of ionic crosslinks; drying the rubber intensifies the ionic interactions and effectively increases the apparent crosslink density, thereby reducing the rate of relaxation.

Commercial natural rubber is produced in different grades; this arises from the different sources of latex coagulum and different methods of handling rubber. Conventional grades (*e.g.* Standard Malaysian Rubber) are processed by coagulating the latex using acids followed by washing and drying the rubber, whilst the processing of deproteinised rubber (DPNR) involves an extra step whereby the naturally occurring proteins are largely removed prior to coagulation[1,2]. Hence, DPNR contains lower amounts of nitrogenous non-rubber constituents than conventional grades.

It has been reported that rubber which has been processed in different ways show a marked variation in properties due to the presence of different types and/or amount of non-rubber constituents. For instance, proteins are believed to affect creep; Smith observed that the removal of proteins from DPNR reduces the rate of creep[3]. Knight and Tan[4] reported that the presence of proteins caused the modulus of rubber vulcanisates to increase, while amino-acids affect the storage hardening of the raw rubber[5]. Storage hardening is also affected by inorganic materials[6]. Certain nitrogenous bases and fatty acids take part in the sulphur vulcanisation reaction which subsequently affects the properties of rubber vulcanisates[7]. The presence of non-rubber constituents also affect the sensitivity of the rubber to water, thereby properties such as elastic modulus and stress relaxation[8].

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The change in modulus with humidity has been reported in earlier publication[9]. This paper gives further experimental results and discusses the effect of some non-rubber constituents, particularly proteins and hydrolysed components (amino-acids) on the stress relaxation behaviour (physical) of unfilled NR vulcanisates. The discussion of the effect of water/humidity on the elastic modulus preceded stress relaxation because the latter is known to be strongly influenced by humidity[10].

EXPERIMENTAL

Natural rubber containing different types and proportions of naturally occurring non-rubber constituents was used for this study. They were purified rubber containing different types of non-rubber constituents; total solid rubber (TSR or generally known as latex film); centrifuged latex fractions; commercial grade Standard Malaysian Rubber (SMR) and deproteinised natural rubber (DPNR). All tests were carried out in duplicate (unless otherwise indicated) and the average readings were taken.

Preparation of Rubber

Total solid rubber (TSR). Total solid rubber was obtained by film drying the fresh latex onto glass plates at room temperature (21°C). The latex film was about 1 mm to 2 mm thick and the drying process took about 24 h – 48 h. A fan was used to speed up the drying process to overcome the problem of bacterial action that would occur in the latices if they were exposed unnecessarily long in an open environment.

Centrifuged fractions. Fresh latex was ultracentrifuged at 19 500 r.p.m. for about an hour in a Beckman centrifuge to give four main fractions: a white rubber fraction (*i.e.* rubber phase); a yellowish-orange layer containing the Frey Wyssling particles; a serum fraction and a grey-yellow gelatinous bottom fraction *(Figure 1)*. They were manually separated to give fractions containing different types and proportions of non-rubber constituents. The rubber phase (RP) was redispersed in distilled water and film dried at room temperature in a similar manner to the TSR. Rubber phase containing bottom and serum fractions were prepared by mixing it with those fractions before the filming and drying process.

Purified rubber (PR). Purified rubber was obtained from the rubber fraction. This was first isolated and then redispersed in 5% aqueous sodium dodecyl sulphate for about 24 h before being recentrifuged to remove the remnants of the serum fractions. The treated rubber phase was rewashed with aqueous sodium dodecyl sulphate followed by water before it was finally redispersed in water to give a purified latex. The purified latex was subsequently film dried on glass plates at room temperature (21°C) to give PR.

SMR L and DPNR

Standard Malaysian Rubber grade L (SMR L) and deproteinised natural rubber (DPNR) were commercial rubber grades.

Isolation of Non-rubber Constituents

Isolation of bottom protein (β-serum protein). The β-serum protein (later termed bottom protein) was obtained from the bottom fraction of the ultracentrifuged fresh latex. The bottom fraction was first freeze-thawed three times, recentrifuged and the clear serum collected. Ammonium sulphate was added to the serum to a saturation level and the mixture was left in the refrigerator for about 6 h. The precipitated protein was collected from the recentrifuged mixture, redissolved in water and dialysed against water to remove any ammonium sulphate remnants. The dialysed mixture was then freeze-dried to give a powdered form of ammonium sulphate precipitated bottom protein.

Isolation of serum protein. The serum protein was obtained from the serum fraction of the ultracentrifuged latex in a similar manner to the bottom protein, through precipitation with ammonium sulphate.

Isolation of proteolipid. The proteolipid was obtained from the rubber phase of the NR latex according to the procedure reported by Hasma[11]. The rubber phase was redispersed in water, filtered and added drop-wise to about five volumes of a continuously stirred chloroform/methanol (2:1, volume/volume) mixture. The extract was separated from the rubber coagulum and washed with salt solution. A lower layer of the chloroform fraction and a thin whitish interfacial layer were isolated. The chloroform layer was concentrated on a rotatory evaporator. The insoluble portion containing proteolipids was collected.

Amino-acids and natural rubber serum powder (NRSP). Amino-acids were of commercial grade and the NRSP was obtained from the Rubber Research Institute of Malaysia pilot plant.

Re-incorporation of Proteins and Amino-acids

Proteins and amino-acids were dissolved in water and then added to the purified latex. The mixture was thoroughly mixed before film drying on glass plates at room temperature. Dried latex films were then blended by using a two-roll mill to give rubbers containing the required proportion of the non-rubber constituents.

Compounding and Vulcanisation

Dried films of purified rubber containing non-rubber constituents, total solid rubber, different fractions of centrifuged latex and commercially processed rubbers (SMR L and DPNR) were mixed in accordance with the formulation given in *Table 1*.

All purified rubbers were mixed in accordance with ACS-1 formula while the commercial grade and total solid rubbers were mixed using the CBS/S systems.

The mixing process was carried out using a laboratory two-roll mill. Moulding of rubber test pieces (1 to 2 mm thick) was carried out using a steam-heated press at 150°C for a period required to fully vulcanise the rubber (*i.e.* t_{100}) measured by a rheometer. Extra care was taken during handling and storage of vulcanisates to reduce degradation since antioxidant was not incorporated.

Test Methods

Relaxed modulus test. The relaxed modulus test (MR 100) was carried out by extending a square-end dumb-bell test piece to 100% extension and measuring the load after one minute. The test was carried out on the rubber conditioned under three different environments.

Stress relaxation measurements. Stress relaxation tests were carried out under tensile deformation. Sample strips (1.0 to 2.0 mm thick) were die-stamped from moulded sheet. Two techniques were employed for this study: a conventional technique and the 'bow string' technique.

TABLE 1. RUBBER FORMULATIONS (P.H.R.)

Vulcanising systems	ACS-1	Conventional[d]	Semi-EV[d]	EV[d]
Rubber[a]	100.0	100.0	100.0	100.0
Zinc oxide	6.0	5.0	5.0	5.0
Stearic acid	0.5	2.0	2.0	2.0
Sulphur	3.5	2.5	1.5	0.6
MBT[b]	0.5	–	–	–
CBS[c]	–	0.6	1.5	2.5

[a] The non-rubber constituents were added at 1% wt. of NR. The nitrogen and ash contents were determined in accordance with *ISO 1656* (1988) and *ISO 247* (1980), respectively

[b] 2-Mercaptobenzothiazole

[c] N-cyclohexyl benzothiazole-2-sulphenamide

[d] $\sqrt{(\mathrm{CBS})\times(\mathrm{sulphur})}$ was kept constant to obtain the same crosslink density

The conventional technique uses modern tensile equipment. Samples were held firmly in screw-tight grips and pulled to a required extension at 100% per minute. The change in stress with time at constant strain was monitored using the output facilities available (*i.e.* a portable computer).

The 'bow string' technique is a new technique of measuring stress relaxation of rubber and detail procedures has recently been published[12]. This technique allows the change in stress, under different environments, to be monitored for longer period of time (>2 weeks).

The new technique of measuring stresses involve pushing/pulling vertically an extended piece of rubber strip at the mid-point to give a three-point bending or 'bow-string' configuration. The stresses at three different angular displacements, namely (about) 4, 6, 8 degrees were calculated and the average value taken.

With both techniques, the sample used were die-stamped from moulded sheet of about 1.0 to 2.0 mm thick and 10 mm wide. Most of the relaxation tests were performed at 30% elongation and extension rate was 100% per minute, unless otherwise stated. At low strain (<50%), the rubber is within the so-called 'affine deformation' region, where the bulk deformation of the sample is considered to be a good approximation to the infinitesimal deformation of the rubber network. Thus, at this low extension, the possible effects due to non-affine deformation will be minimised.

The results were either presented as a plot of relative stress, which is the ratio of stress to the reference stress, against log(time) or as relaxation rate. The rate was calculated from the stress *versus* log(time) plot and expressed as percent stress relaxation per decade of time.

Conditioning samples at different relative humidities. The relaxation test was carried out at three different relative humidities, namely low humidity (7% to 25%RH), room humidity (50% to 60%RH) and high humidity (100%RH). Prior to the test, the sample strips which were die-stamped from moulded sheet, were conditioned at the respective humidity for about 14 days.

The low humidity environment was achieved by placing phosphorus pentoxide (P_2O_5) in a desiccator. Samples were placed in the desiccator and taken out only during testing.

The higher humidity condition was obtained by immersing the sample in water. It was carried out by placing the sample in water for between 14 to 30 days. The change in weight of the sample due to absorption of water into the rubber was measured prior to testing.

Equilibration of samples at room humidity was carried out by placing the rubber strip in a dark cupboard. The relative humidity varied from about 48%RH to about 60%RH, depending on the outside weather.

Crosslink density measurement. The crosslink density of rubber was determined from equilibrium swelling data using the Flory-Rehner equation[13]. Samples of about 2 mm thickness were cut into 25 mm squares and swollen in toluene in the dark for several days, during which their weights were regularly monitored. At equilibrium swelling, the weights were recorded and sample dried in a vacuum oven at 60°C. The difference between the weight of the swollen and dried samples was taken as the true weight of the solvent imbibed.

RESULTS AND DISCUSSION

Physical (or primary) relaxation has been associated with movement taking place amongst the following: rubber network; the side groups on the rubber chain; chain entanglements and filler structures/particles. This type of relaxation is dominant in the early stages of the relaxation process ($<10^4$ min), particularly at ambient or low temperatures and the changes in stress has been observed to be linear with log(time)[8,14].

Effect of Non-rubber Constituents

It has been reported that DPNR has greater resistance to relaxation than conventional grades of SMR[3]. The current work which was carried out using three different types of rubber containing different proportions of natural non-rubber constituents is in agreement with those results. They were the unfilled DPNR, a conventional grade NR (SMR L) and TSR vulcanised using a semi-EV system; TSR has a higher proportion of non-rubber constituents than SMR L and DPNR has the lowest content.

The results, presented as a plot of relative stress, f/f_0 against log t are given in *Figure 2*. DPNR was observed to have a lower rate of physical stress relaxation than SMR L or TSR. The stress relaxation rate of TSR was marginally higher than that of SMR.

The lower rate of stress relaxation of DPNR compared to SMR and TSR is presumably due to the removal of non-rubber constituents during the deproteinisation process. The non-rubber constituents removed during the production of DPNR include proteins and amino-acids. A study was carried out to relate the presence of these individual non-rubber constituents to the relaxation rates of the rubber. Initially, tests were carried out by using three different centrifuged fractions of latex, namely the rubber phase (RP), the RP+bottom fraction, and the RP+serum fraction. Results showed that the magnitudes of the rates of relaxation of those rubbers are as follows (*Figure 3*):

> (RP=RP+bottom fraction) < (RP+serum fraction) < TSR

The relaxation rate of the RP containing added serum fraction is about 2.7% per decade, which is approximately the same as the TSR.

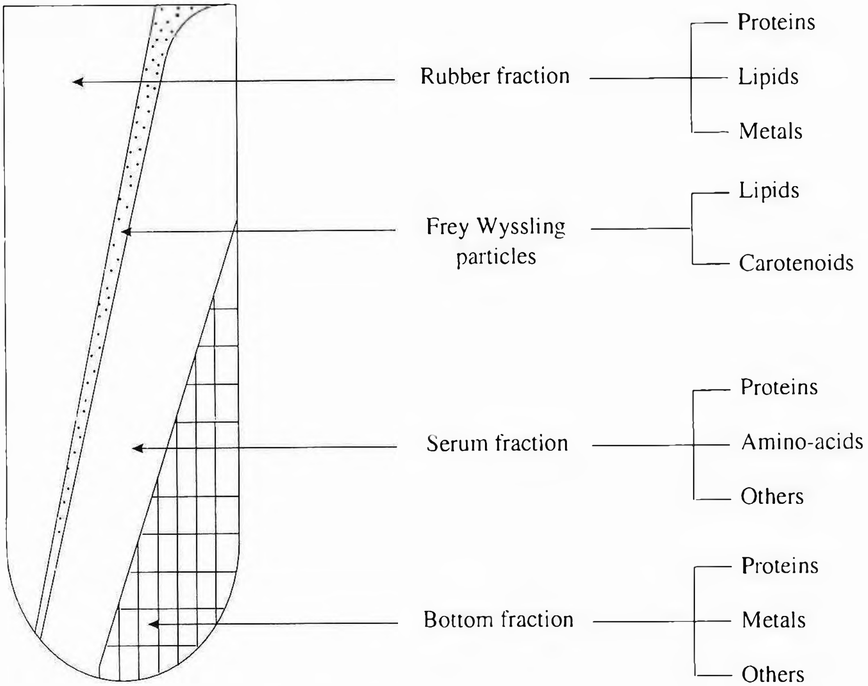


Figure 1. Ultracentrifuged fresh natural rubber latex fractions.

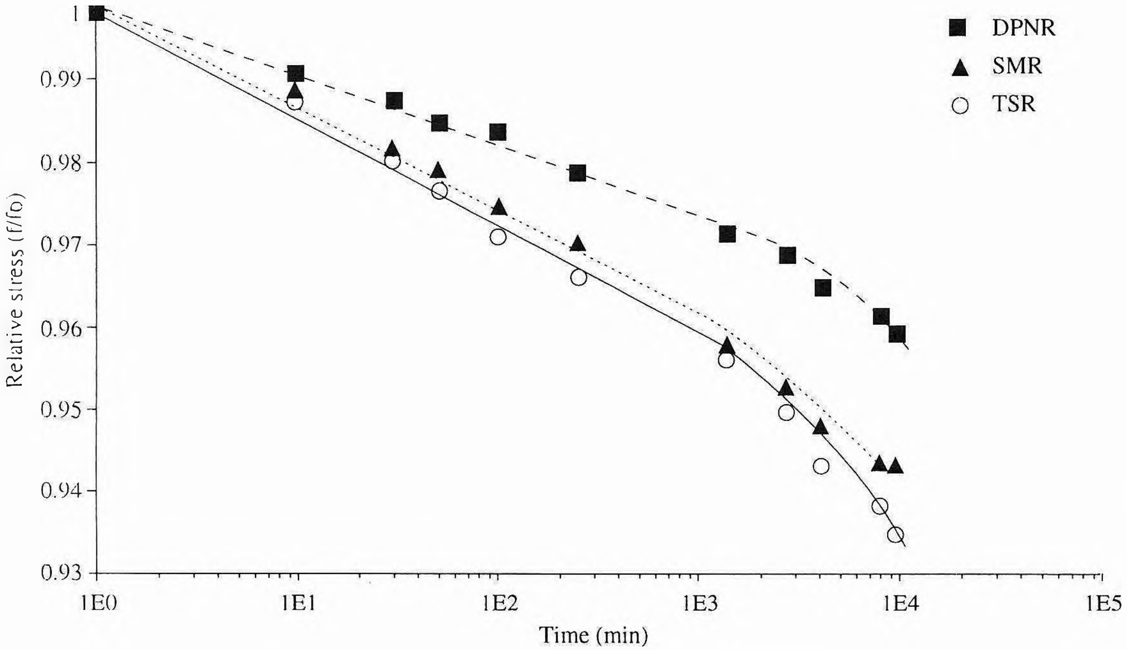


Figure 2. Stress relaxation of different types of natural rubber (Semi-EV formulation; 55%RH).

This shows that most of the non-rubber constituents which increase the stress relaxation of the rubber are in the serum rather than the bottom fraction.

The serum fraction contains several types of non-rubber including proteins and amino-acids. Proteins are the major component of the non-rubbers and amino-acids are their hydrolysis products. Accordingly, the effects of proteins and the amino-acids on stress relaxation were investigated. Results presented as a plot of relative stress against log(time) are shown in *Figure 4*.

The incorporation of 1% protein into the purified rubber did not give any significant change to the relaxation behaviour of the NR vulcanisates. The relative stress of purified rubber containing proteins was approximately the same as for purified rubber. A similar value was obtained with amino-acids such as glutamic acid.

However, the incorporation of amino-acids such as alanine and arginine, gave a different result; their relaxation rates were markedly lower. The presence of 1% wt. alanine and arginine reduces the relaxation rate of purified rubber by about 30% and 60%, respectively.

It may be noted that there are differences in the rate of relaxation between two 'clean' rubbers: purified rubber (*ca.* 0.04% wt. nitrogen) and DPNR (*ca.* 0.08% wt. nitrogen). It would be expected that these two rubbers have approximately similar relaxation rates due to the low amount of nitrogenous materials. However, direct comparisons of the results in *Figures 2* and *4* show the rate of relaxation of DPNR is lower than the rate for purified rubber.

This difference could be due to three possible reasons. Firstly, the two rubbers were processed by different methods, resulting with rubber having different average molecular weight. Purified rubber possesses lower molecular weight than DPNR as reflected by the lower Mooney viscosity values (*Table 2*). This could lead to purified rubber having comparatively higher relaxation rate than DPNR.

TABLE 2. MOONEY VISCOSITY OF RAW RUBBER

Rubber	Mooney viscosity [ML(1+4)@100°C]
Purified rubber	64
DPNR	81

Secondly, the two rubbers were compounded using different formulations–DPNR using a semi-EV and purified rubber with the ACS-1 formulation. This will give rubber having different types of crosslink and give rise to differences in the relaxation rate.

Thirdly, commercial grade DPNR is treated routinely with thiourea after the coagulation and washing processes. Thiourea treatment is carried out to increase the plasticity retention index of raw rubber; an index giving a measure of the oxidisability of rubber. This occurs by the chelation of the pro-oxidants present in the rubber and with the introduction of small amount of crosslinks to the rubber network. This treatment will result in DPNR having higher resistance to physical relaxation. No thiourea treatment was given to purified rubber.

These three different processes give two different types of rubber which could not be compared directly and the differences in the relaxation properties between DPNR and purified rubber could possibly be due to one/more of these factors.

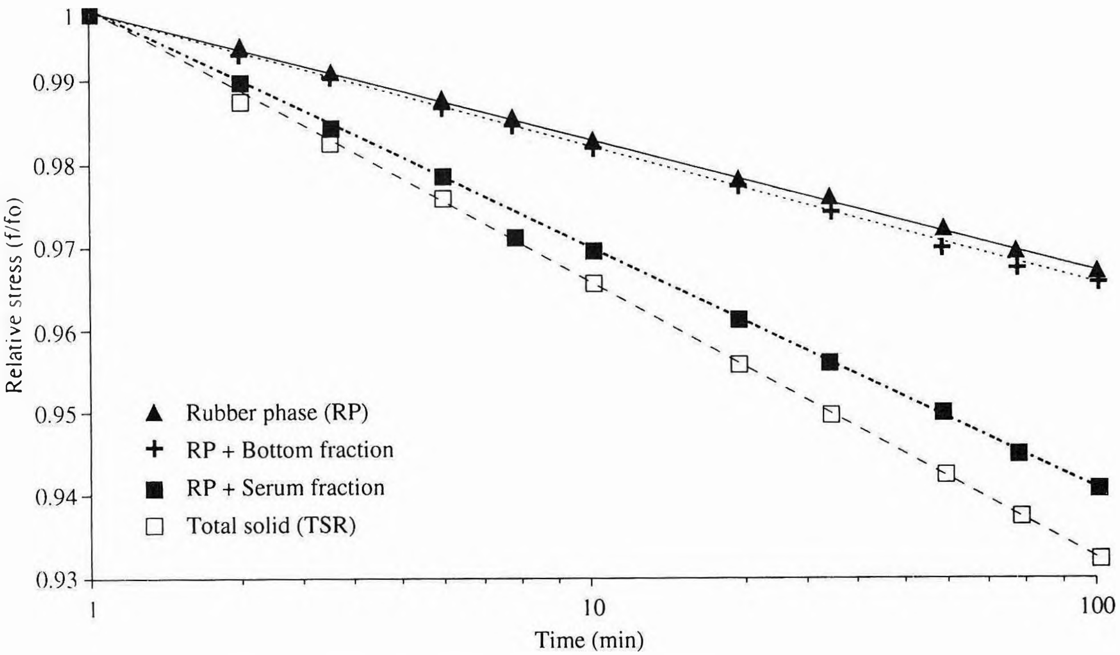


Figure 3. Stress relaxation of centrifuged natural rubber latex fractions (ACS-1 formulation; 55%RH).

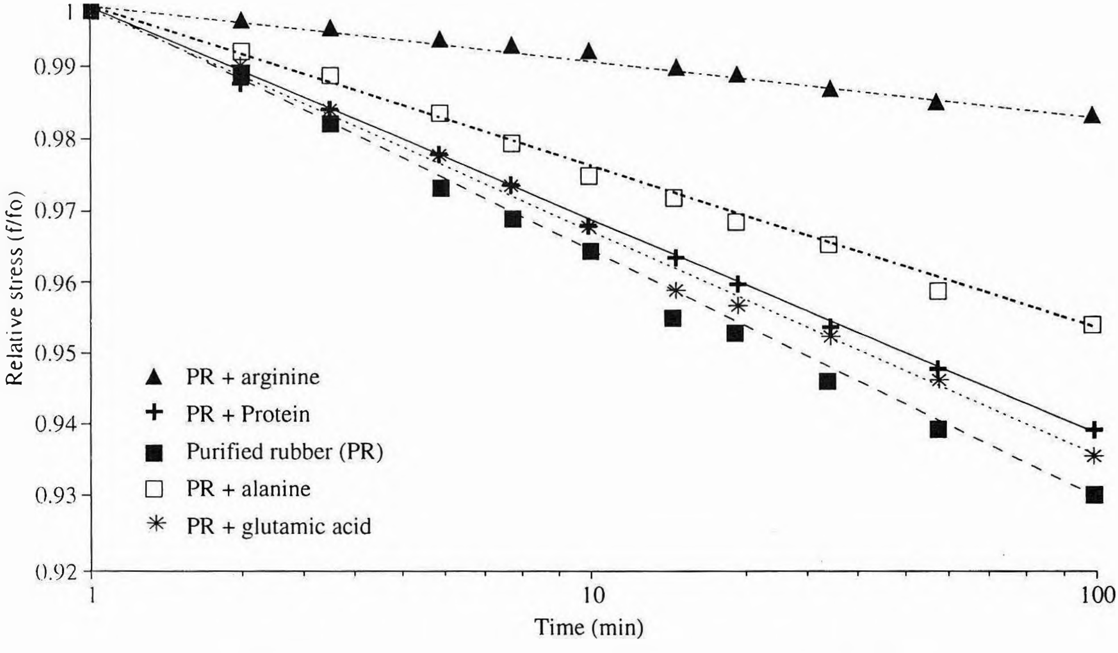


Figure 4. Stress relaxation of purified rubber (PR) containing proteins and amino-acids (ACS-1 formulation 55%RH).

Physical stress relaxation is due to physical realignment and chain slippages. Any factor which influences chain mobility will affect the rates of physical relaxation. This includes internal lubricants, molecular chain modifications and/or changes in the crosslink density.

Previously[9], it was shown that the presence of alanine and arginine increases the crosslink density of rubber. The presence of these amino-acids was shown earlier to give rise to rubber having a good resistance to physical relaxation. Thus it is likely that the good resistance to stress relaxation of these rubbers could be due to the increase in the crosslink density and/or decrease in the polysulphidics rank; that is, the presence of those non-rubber constituents resulted in rubber having a higher crosslink density, thus better resistance to relaxation.

The dependence of relaxation rate on crosslink density was clearly shown when the rate was presented as a function of molecular weight between crosslinks, M_c , which is inversely proportional to crosslink density.

Results showed that the relaxation rates of several purified rubbers containing proteins and amino-acids were linearly related to the M_c (*Figure 5*). This linear relationship shows that the relaxation rate decreases as the crosslink density of the rubber increases. This is consistent with published results which showed a linear decrease in relaxation rates as the crosslink density increases[10].

Thus, it is most likely that the improvement in the relaxation behaviour of purified rubber is due to the increase in crosslink density and/or reduction in polysulphidics rank. This occurs, possibly, during the vulcanisation reaction due to the presence of amino-acids such as alanine and arginine. The presence of these extra crosslinks reduces the ability of the chain movement and slippages, thus lowering the relaxation rates.

Effect of Humidity

Types of rubber. In this study, the effect of humidity on the stress relaxation rate of different types of NR was demonstrated. Rubber containing different amounts and types of non-rubber constituents was used. They include TSR, SMR, DPNR and purified rubbers containing added proteins and amino-acids.

Results obtained on tests carried out with various rubbers are given in *Figure 6*. Generally, it was observed that rubber containing higher non-rubber constituents (TSR) gave larger changes in relaxation rates than 'cleaner' rubber (DPNR). Hence, the presence of non-rubber constituents can cause the larger variability in the relaxation rates of TSR compared to DPNR due to changes in relative humidity.

When the effects of proteins and amino-acids were studied, small variation (<8%) in the relaxation rates was observed (*Figure 7*). For instance, with purified rubber containing 1% wt. arginine, the relaxation rate varies from about 1.26% to 1.33% per decade when the relative humidity was changed from 25% to 100%. The presence of proteins gave a slightly larger change in relaxation rates with humidity.

These results suggest that the non-rubber constituents which give a large variation in relaxation rates with humidity are not amino-acids; amino-acids increase the crosslink density, and improves the resistance to physical relaxation, thus reducing the variability at

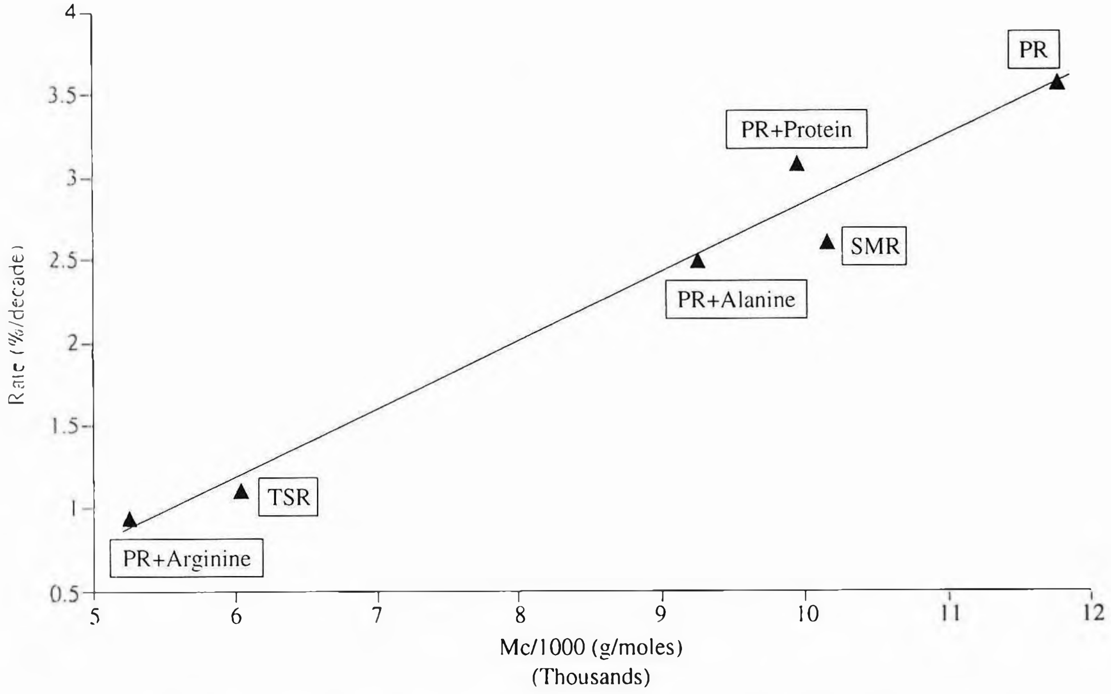


Figure 5. Stress relaxation rates as a function of molecular weight between crosslink (Mc).

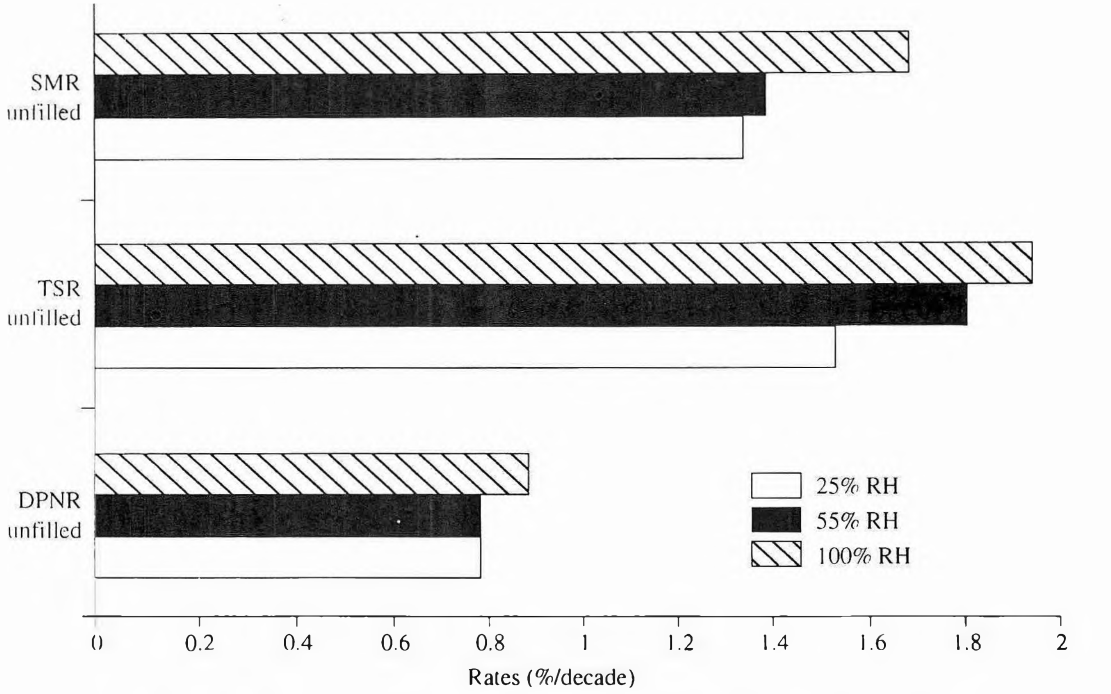


Figure 6. Stress relaxation rates of different types of rubber (Semi-EV formulation).

different humidities. So some other factors must be causing the changes.

It is possible that the non-rubber constituents in TSR which cause the large variation in relaxation rates are the materials which are chemically inactive and behave as inert fillers. Protein is one such material. In this study, the variation observed with purified rubber containing proteins was small; though larger than the change observed with the presence of amino-acids. The amount of proteins added (1% wt.) may be too low to give a more significant variation. If we were to assume that the increase in rate with humidity is linearly related to the amount of proteins, then when the humidity changes from 25%RH to 100%RH, we would obtain about 21% increase in rates when the protein content is increased to 3% wt., the approximate amount present in TSR. However, we do not have any evidence for linearity to date so it would be wise to consider what else could have caused the changes in relaxation due to moisture uptake.

Muniandy and Thomas[15] reported that the presence in rubber of low molecular weight inorganic materials increased the affinity to water. Low molecular weight/hydrophilic materials, (which are inorganic salts) are known to be present in TSR and commercial grades rubber, but not in purified rubber. Thus it is likely that the large changes in relaxation rates obtained with those rubbers are due to the presence of those inorganic materials.

The change in modulus with humidity was found to be independent of the type of crosslink present. This is shown by TSR vulcanised using conventional, semi-EV, EV and peroxide systems, in which the modulus of the rubber consistently decreased by about 22% when the relative humidity was changed from 7% to 55% (*Figure 8*).

Correlation was observed between the increase in crosslink density and the changes in modulus with humidity. That is to say, the presence of the non-rubber constituents which cause the increase in the crosslink density of the vulcanisates gave the biggest change in the modulus with the change in the relative humidity. Thus, certain types of amino-acids such as arginine and alanine gave a larger reduction in modulus with humidity changes compared to the presence of other non-rubber constituents. These amino-acids are present in the serum fraction of NR latex[16].

The reduction in modulus when the rubber is being equilibrated at higher relative humidities is reversible. This is clearly shown by unfilled TSR which was subjected to a series of humidity cycles from 55% through 7%, 100% and back to 55% (*Figure 9*). The initial modulus obtained at 55%RH was observed to remain approximately the same at the end of the humidity cycles.

The change in modulus was observed to be very marked when the sample was exposed to moisture. The variation was observed to be significant in the presence of alanine and arginine, the two amino-acids present in the serum fraction of the NR latex. There was no change in the mass water uptake between rubber equilibrated at low (7% to 25%RH) and room humidity (55%RH). A similar effect was observed with the physical stress relaxation behaviour of the rubber equilibrated at different relative humidities.

The reduction in modulus and increase in rates of relaxation due to the presence of water could be explained by the plasticising effect of the water, an effect similar to the presence of oily plasticiser in rubber[17]. The plasticising effect of water lowers the modulus because the rubber network structure approaches a more

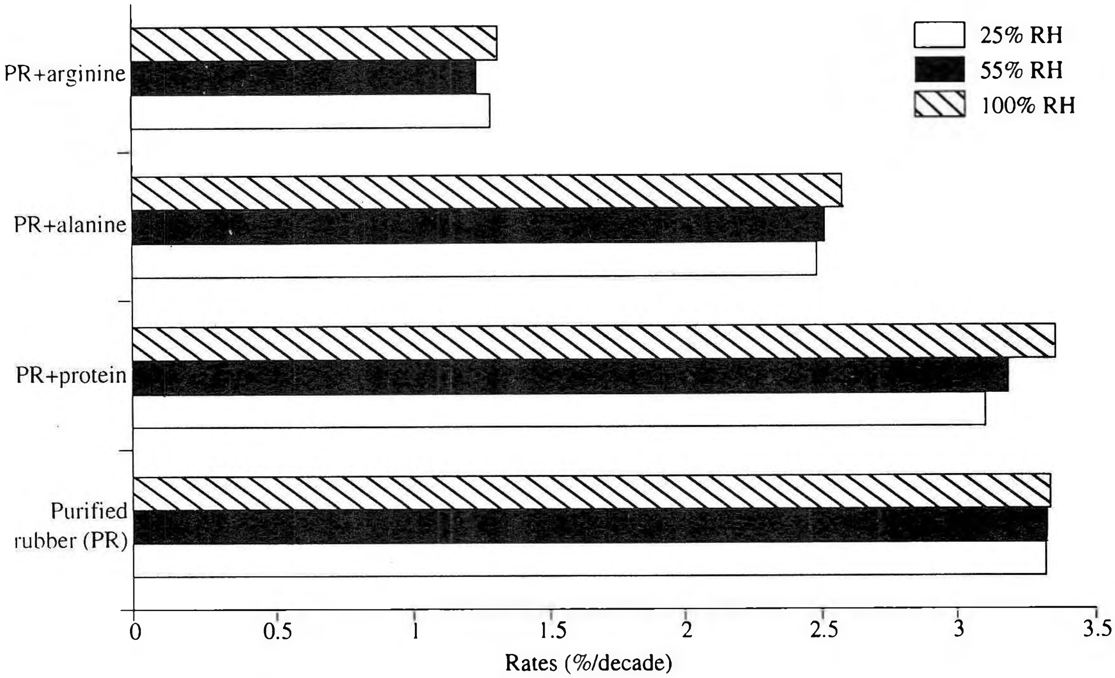


Figure 7. Stress relaxation rates of purified rubber (PR) containing non-rubber constituents (ACS-1 formulation; 55%RH).

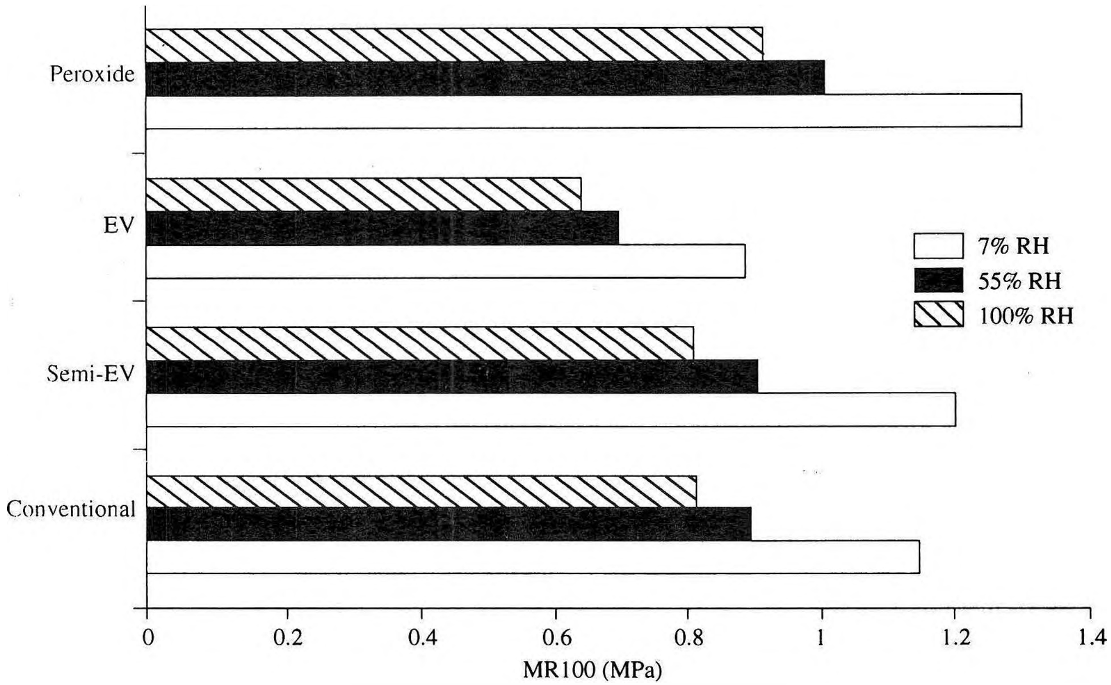


Figure 8. Change in modulus with humidity for TSR.

nearly equilibrium configuration. This being so, it would be expected that the subsequent rate of the 'wet' rubber would be lower. However, it was observed that the rate of relaxation of unfilled NR increases as the amount of water imbibed increases. This is shown in *Figure 10* where the relaxation rates of NR (SMR L) vulcanisate swollen in water is presented as a function of applied strain. The rates of relaxation clearly increased as the water content in the rubber increased.

These results indicate that the explanation for reduction in modulus and increase in relaxation rates with increasing relative humidity is more complicated than the 'plasticisation' theory which suggests that the presence of water 'plasticises' the rubber network. If the 'plasticisation' theory is valid, we would expect the differences in modulus and relaxation at humidities between 7%RH and 55%RH to be negligible since there was no difference in the uptake of water by the rubber at those humidities. However the above results showed that a much bigger effect could be obtained when humidity was reduced from 55%RH to 7%RH; increasing the relative humidity to 100%RH gave a comparatively smaller effect.

Thus the 'plasticisation' theory could not adequately explain the phenomena observed in this study. A possible and more likely process which has taken place is a reaction similar to the phenomenon of storage hardening of unvulcanised raw rubber[18,19]. The presence of certain types of amino-acid may catalyse the formation of active functional groups from the available side or abnormal groups on the rubber chains, namely the epoxide or the carbonyl groups (*Scheme 1*).

The presence of the –OH group and the –COOH groups may result with the following crosslinking esterification reaction[19] (*Scheme 2*).

However, the above esterification process is not reversible and this is not consistent with the current observation whereby the effect of the modulus on humidity is reversible. Thus, a plausible reaction which may give a reversible effect and yet provide a strong linkage may be the ionic crosslink. The crosslink may be catalysed by metal ions (which are present in the rubber) or the amino-acid zwitter ions (*Scheme 3*).

Such systems are known to be sensitive to moisture – this is explicable, for a strong hydrated ion reduces the strength of the crosslink. Conversely, drying the rubber reduces the solvation by water, intensifies the ionic interactions and hence effectively increases the apparent crosslink density and reduces relaxation rate.

An alternative explanation (for S_8-cured rubber) would be the ionic polysulphidic exchange reactions between crosslinks:

$$R_1{-}S_x{-}S_y{-}R_2 + R_3{-}S_{z-1}{-}S^- \leftrightarrow R_1{-}S_x{-}S_z{-}R_3 + R_2{-}S_{y-1}{-}S^-$$

The relaxation rate would increase with ionic strength. However, since the effect was also observed with peroxide cured rubber, the latter explanation may be unjustified.

CONCLUSIONS

Under ambient conditions, wide variation in the modulus and stress relaxation was observed when non-rubbers were present. Much relates

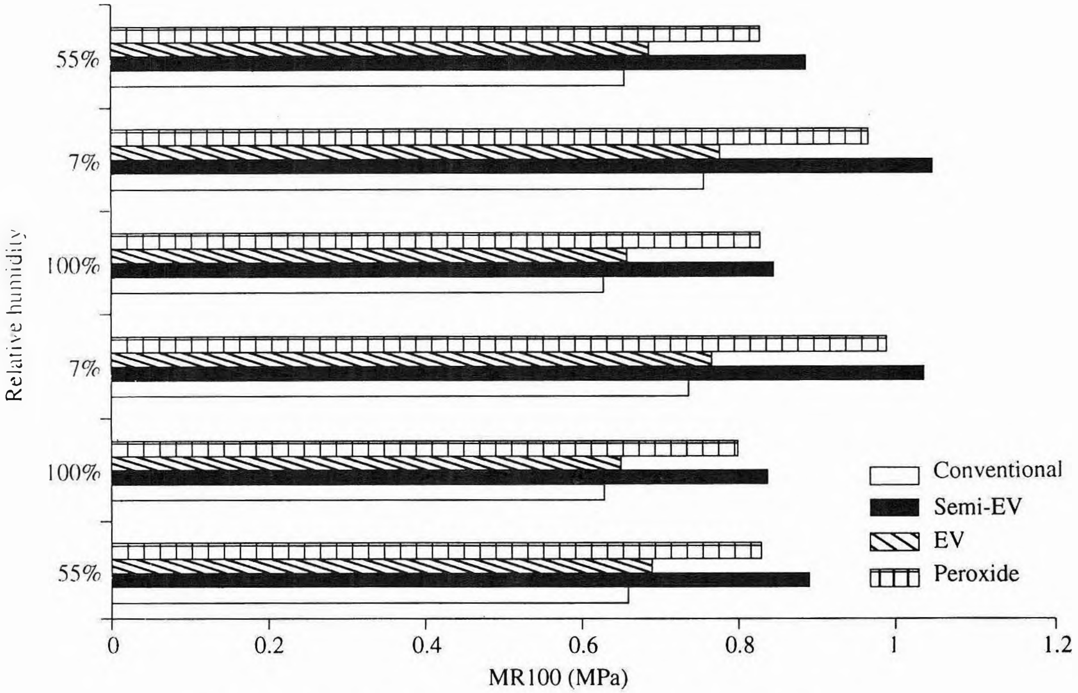


Figure 9. Change in modulus of TSR after a series of humidity cycle.

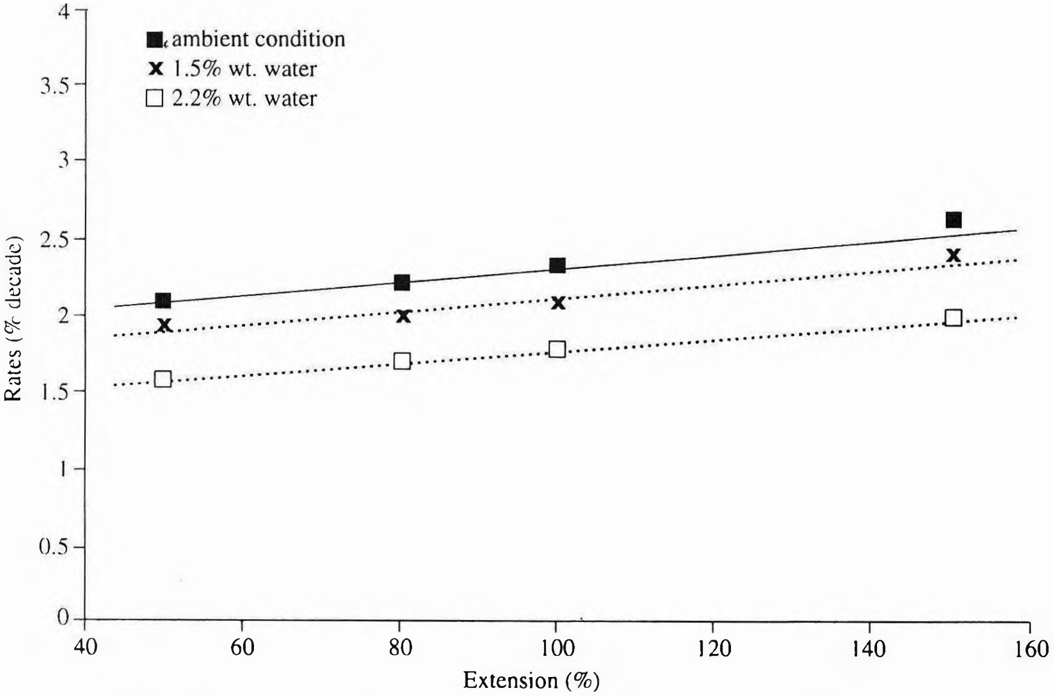
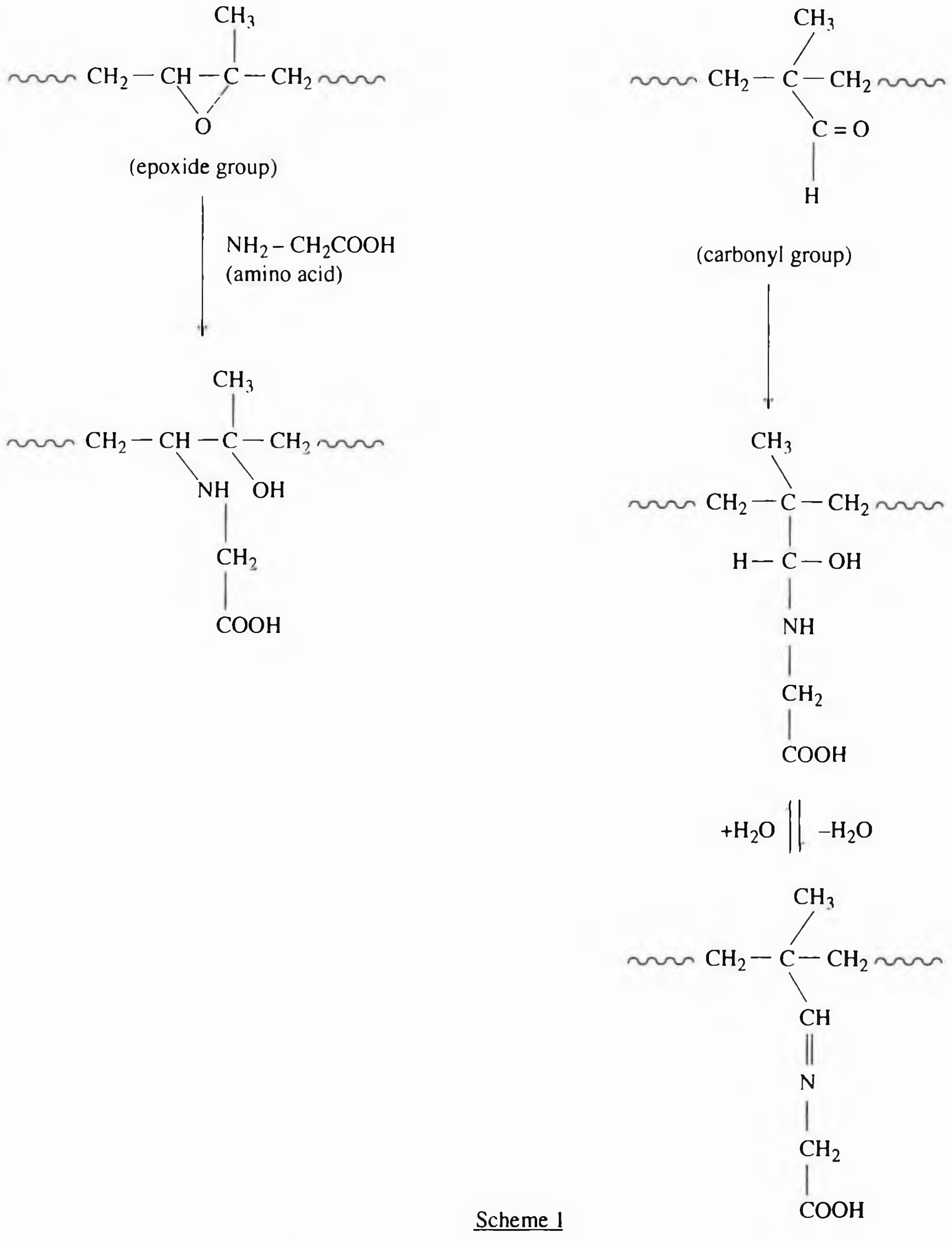
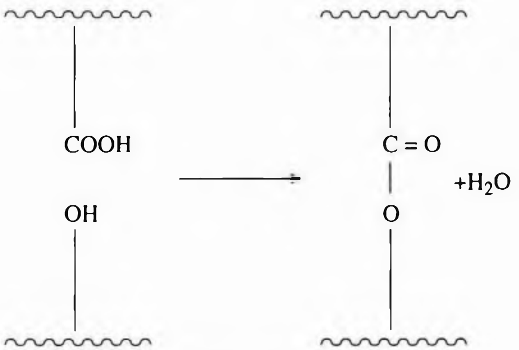


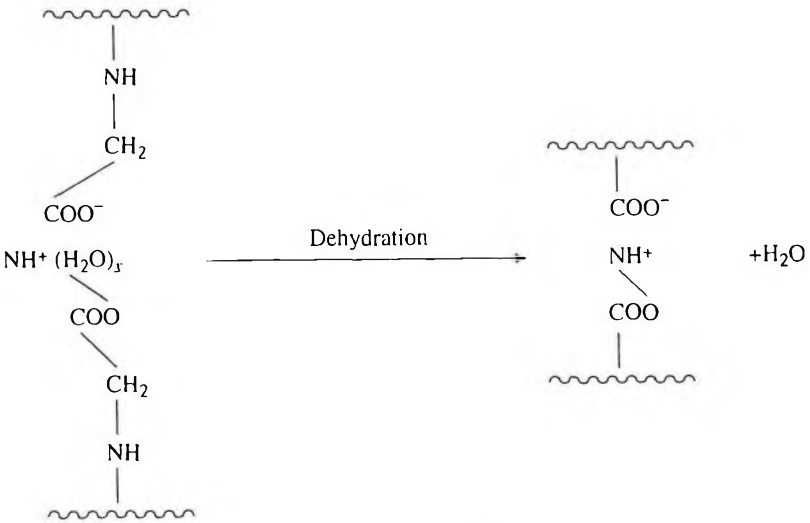
Figure 10. Stress relaxation rates of 'wet' natural rubber (Semi-EV formulation).



Scheme 1



Scheme 2



Scheme 3

to the presence of amino-acids, particularly, alanine and arginine, which resulted in an increase in modulus and reduction in relaxation rates. Proteins did not significantly affect these properties; they acted as inert fillers.

At lower humidity, the relaxation rate is lower than at higher humidity. The modulus is higher at lower humidity.

The largest variation in properties was caused by the presence of amino-acid. It was reasoned that these variations were due to the formation of ionic crosslinks.

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Effects of Tapping and Intensive Stimulation on Yield, Dryness Incidence and Some Physiological Latex Parameters of Clone RRIM 600

DO KIM THANH*, S. SIVAKUMARAN**# AND WONG KAI CHOO***

A four-year experiment studied the yield responses, dryness incidence and some physiological latex parameters of clone RRIM 600 on two tapping frequencies (alternate daily d/2; fourth daily d/4) of half-spiral tapping system combined with four stimulation frequencies [unstimulated control (0/y); 4 applications/year (4/y); 30 applications/year (30/y); 60 applications/year (60/y)] of 2.5% ethephon applied by groove method. Low tapping frequency of d/4 gave significantly higher g/t/t but lower kg/ha/year and cumulative kg/ha when compared to d/2 frequency. When very high stimulation frequency of 30/y or 60/y were applied, the bulk of the yield increase in response to stimulation was only recorded in the first year of tapping, with increase thereafter being marginal when compared to the unstimulated control. No significant differences in yield were obtained between stimulations 30/y and 60/y. Incidence of dryness was not affected by stimulation frequency upto 30/y, but was significantly increased when at stimulation frequency of 60/y.

Trees tapped on d/2 frequency in contrast to d/4 recorded consistently lower readings of physiological latex parameters such as plugging index (PI), initial flow rate (IFR), total solid content (TSC) and dry rubber content (DRC) but higher bottom fraction (BF), thiol content (R-SH) and inorganic phosphorus content (Pi). Similarly, intensive frequencies of stimulation (30/y and 60/y) produced lower values of PI, IFR, TSC and DRC but higher values of BF and Pi when compared to low stimulation frequency 4/y or unstimulated control. Under our experimental conditions sucrose and pH of latex were not affected by both tapping and stimulation treatment and this finding is at variance with other published reports. The significance of changes in these various physiological latex parameters are discussed in relation to yields obtained with the different combinations of tapping and stimulation frequencies.

The yield productivity of *Hevea* trees can be increased beyond its genetic potentials by appropriate manipulation of tapping and stimulation methods. However, very intensive tapping could lead to physiological stress in the tapping panel resulting in development of

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tapping panel dryness[1,2]. Similarly heavy stimulation over the long term on successive tapping panels could also result in deleterious effects on the physiology and anatomy of the bark[3].

It is imperative for sustained yield productivity of *Hevea* trees that the exploitation intensity adopted does not lead to irreversible physiological stress in the tapping panel. It has been shown that the physiological status of the tapping panel can be monitored by routine analysis of selected physiological latex parameters[4]. It was therefore considered beneficial to study changes in physiological latex parameters in trees subjected to various intensities of exploitation. Thus in this study changes in some physiological latex parameters in trees of clone RRIM 600 subjected to two tapping systems in combination with intensive stimulation have been investigated. The relationship between changes in these parameters and yield and associated parameters are discussed.

MATERIALS AND METHODS

The experiment was conducted at *Field 53C*, Rubber Research Institute Experiment Station, Selangor, Malaysia, on clone RRIM 600. The trees were tapped on half-spiral system with two tapping frequencies, *viz.* alternate daily tapping (d/2) and fourth daily tapping (d/4). These tapping systems were combined with stimulation frequencies of 0, 4, 30 and 60 applications per year, *viz.*:

- ½S d/2 6d/7 unstimulated (control);
- ½S d/2 6d/7+ET 2.5% Ga(1) 4/y (4 applications a year)
- ½S d/2 6d/7+ET 2.5% Ga(1) 30/y (30 applications a year)
- ½S d/2 6d/7+ET 2.5% Ga(1) 60/y (60 applications a year)
- ½S d/4 6d/7 unstimulated
- ½S d/4 6d/7+ET 2.5% Ga(1) 4/y (4 applications a year)
- ½S d/4 6d/7+ET 2.5% Ga(1) 30/y (30 applications a year)
- ½S d/4 6d/7+ET 2.5% Ga(1) 60/y (60 applications a year).

Stimulation was by means of ethephon at 2.5% concentration applied by the groove method. Yield recordings were carried out on second virgin panel (*BO-2*). The duration of recording was for a period of four years. Physiological latex parameters were determined from March to October 1994, this duration being towards the final year of the four-year period of yield recording. Each treatment was replicated three times with nine trees per replication. The treatments were laid in a randomised complete block design (RCBD).

For yield recording, latex from all trees per treatment per replication were bulked together and weighed as bulked wet weight of latex. Late drips were similarly recorded as bulked wet weight of cuplump. Dry rubber content (DRC) of latex was determined twice a month for each treatment while that of cuplump was calculated on the assumption of 40%–50% water content.

Dryness incidences were recorded in November of each tapping year. The percentage of dryness was derived by expressing the total length of dry cut of each treatment as a percentage of the total length of cut of that treatment.

For determination of physiological latex parameters, the following methods were followed:

- *Initial flow rate and plugging index.* Initial flow rate (IFR) (ml/minute) and plugging index (PI) were determined following the method described by Milford *et al.*[5] IFR defined as the rate of latex flow in millilitres per minute was recorded by measuring the volume of latex at the end of five minutes flow. PI was derived by dividing the IFR by total volume of latex recorded on the day of measurement. This was multiplied by a common factor of 100 for convenient expression.

- *Determination of bottom fraction.* Bottom fraction (BF) (%) was determined by the haematocrit method. Latex was pipetted into a haematocrit tube which had one end sealed. The sample was kept in ice (0°–5°C). It was centrifuged at 15 000 r.p.m. for 15 min using a micro haematocrit centrifuge (MHC-Hawksley). As soon as the sample was taken out of the centrifuge, the length of bottom fraction as distinguished from the top rubber fraction by a layer of clear serum was measured.

BF was calculated as:

$$\text{BF \%} = \frac{\text{Length of BF}}{\text{Total length of latex column}} \times 100$$

- *pH measurement.* pH of latex was measured by a pH-meter (Hanna Instrument 8417) with combined electrode WTM E-50. Prior to measurement, the pH-meter was calibrated with buffer solution of pH = 7.01 at ambient temperature. The electrode was dipped in the latex and after a stabilisation period of 2–5 min per reading was recorded.

- *Other parameters.* Determination of total solid content (TSC), DRC in %, sucrose, inorganic phosphorus (Pi), thiols in mM (R-SH) were carried out following the methods described by I.R.C.A.[6], Jacob *et al.*[7,8], and Do and Nguyen[9].

The sampling procedures for analysis of physiological latex parameters were as follows: 20 drops of latex from each tree in the same replication were pooled into a small vial which was kept in ice. The pooled latex was then pipetted in 1 ml fraction for TSC determination and 1 ml for extraction by 2.5% trichloroacetic acid. The extracted aliquots from trichloroacetic acid was always kept in cooled condition (0°–5°C) for the determination of sucrose, inorganic phosphorus and thiols content.

RESULTS

Mean Dry Rubber Yield per Tree per Tapping

Analysis of variance showed that there were significant treatment but no interaction effects on mean dry rubber yield per tree per tapping (g/t/t) over 4 years. High tapping frequency of d/2 gave significantly lower yield (g/t/t) when compared to d/4 frequency (*Table 1*). All stimulated treatments produced significantly higher g/t/t than the unstimulated control. Among the stimulated treatments, intensive stimulations of 30/y and 60/y produced significantly higher yield than stimulation of 4/y. However, there was no significant difference between 30/y and 60/y.

TABLE 1. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON MEAN DRY RUBBER YIELD PER TREE PER TAPPING (G/T/T) OVER 4 YEARS OF TAPPING

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	57.9	64.7	79.9	77.6	70.0
½S d/4	57.1	70.3	92.5	100.4	80.1
Mean	57.5	67.5	86.1	89.0	75.1

$LSD_{0.05}$ (tapping frequency) = 6.1
$LSD_{0.05}$ (stimulation frequency) = 8.7

The trend of mean g/t/t over four years of tapping is shown in *Figure 1*. There appeared to be differences between the two tapping frequencies in response to stimulation. Under high tapping frequency of d/2, stimulations 30/y and 60/y produced higher yield when compared to stimulation 4/y or the unstimulated control in the first year of tapping. However, their yield differences declined with subsequent years of tapping such that at the fourth year of tapping, no differences in yield were detected among stimulated and unstimulated trees. With low tapping frequency of d/4, the higher yields produced by stimulations 30/y and 60/y over those of stimulation 4/y or unstimulated control were sustained throughout the four years of tapping though their yield differences were less in later tapping years.

Mean Dry Rubber Yield per Hectare per Year

There were significant treatment but no interaction effects on mean dry rubber yield per hectare per year (kg/ha/year) over four years of tapping. High tapping frequency of d/2 produced significantly higher kg/ha/year than the lower tapping frequency of d/4 (*Table 2*). This result was the reverse of that of g/t/t.

All stimulated treatments produced significantly higher kg/ha/year than the unstimulated control. Among the stimulated treatments, stimulations of 30/y and 60/y gave significantly higher yield than stimulation 4/y.

Cumulative Dry Rubber Yield per Hectare

Analysis of variance on cumulative dry rubber yield per hectare (kg/ha) showed significant treatment but no interaction effects. Results on kg/ha cumulated over four years of tapping were similar to those of kg/ha/year (*Table 3*).

Dryness Incidence

The data on frequency of dryness incidence at the end of fourth year of tapping are presented in *Table 4*. Values in percentages were transformed using square root transformation and analysis of variance performed on the transformed values. Significant effect due to stimulation treatment was obtained while there were no significant effects due to tapping frequency or interaction. High stimulation frequency of 60/y resulted in significantly higher dryness incidence than the other stimulation treatments as well as the unstimulated control. No

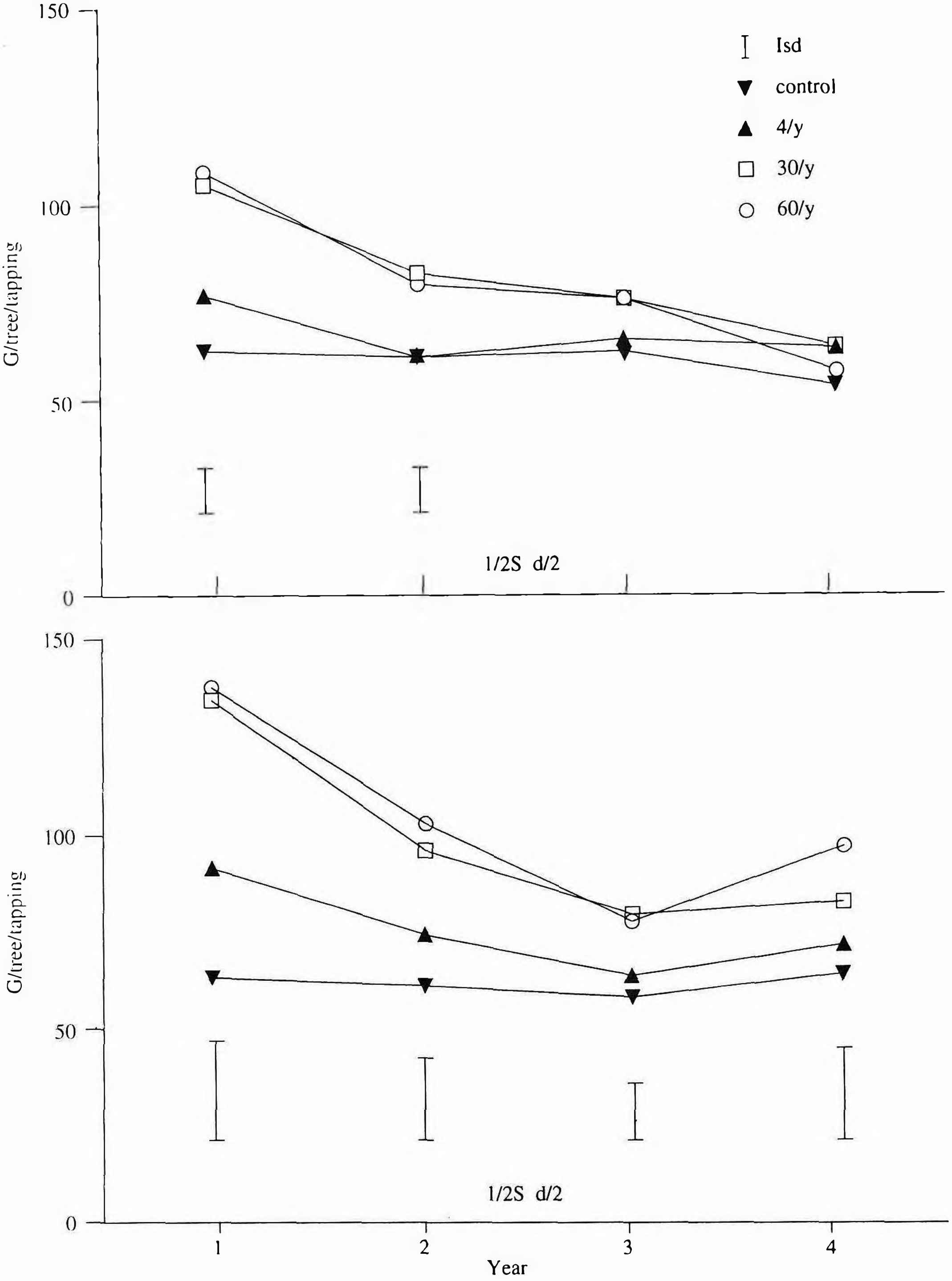

Figure 1. Mean g/t/t as influenced by stimulation treatments under two tapping frequencies.

TABLE 2. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON MEAN DRY RUBBER YIELD PER HECTARE PER YEAR (KG/HA/YEAR) OVER 4 YEARS OF TAPPING

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	2 056	2 298	2 829	2 754	2 484
½S d/4	1 103	1 358	1 786	1 939	1 547
Mean	1 580	1 828	2 308	2 347	2 015

$LSD_{0.05}$ (tapping frequency) =160

$LSD_{0.05}$ (stimulation frequency) = 227

TABLE 3. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON CUMULATIVE DRY RUBBER YIELD PER HECTARE (KG/HA) OVER 4 YEARS OF TAPPING

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	8 223	9 193	11 317	11 016	9 937
½S d/4	4 410	5 429	7 142	7 754	6 184
Mean	6 317	7 311	9 230	9 385	8 061

$LSD_{0.05}$ (tapping frequency) =642

$LSD_{0.05}$ (stimulation frequency) = 907

TABLE 4. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON DRYNESS INCIDENCE

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	8.0 (2.2)	3.8 (2.1)	7.3 (2.6)	22.3 (4.7)	10.4 (2.9)
½S d/4	0.0 (1.0)	3.6 (1.8)	6.4 (2.5)	16.9 (4.1)	6.7 (2.4)
Mean	4.0 (1.6)	3.7 (2.0)	6.9 (2.6)	19.6 (4.4)	8.6 (2.7)

Figures in brackets denote the transformed data

$LSD_{0.05}$ on transformed values (tapping frequency) = 0.8

$LSD_{0.05}$ on transformed values (stimulation frequency) = 1.1

significant differences in dryness incidence were obtained between the lower stimulation frequencies (4/y and 30/y) and the control.

Physiological Latex Parameters

Physiological latex parameters associated with latex flow. Analyses of variance on physiological latex parameters associated with latex flow function such as PI, IFR, DRC, BF and TSC revealed significant effect due to tapping and stimulation treatments but no significant effect due to interaction.

Plugging index. High tapping frequency of d/2 resulted in significantly lower PI than the lower tapping frequency of d/4 (*Table 5*). All stimulated treatments produced significantly lower PI than the unstimulated control. There was a progressive reduction in PI with increase in stimulation frequency from 4/y to 60/y.

Initial flow rate. High tapping frequency of d/2 produced significantly lower IFR than low tapping frequency of d/4 (*Table 6*). Significantly lower IFR were produced by stimulation frequencies of 30/y and 60/y when compared to those of stimulation 4/y or unstimulated control.

Bottom fraction. High tapping frequency of d/2 produced significantly higher BF than low tapping frequency of d/4 (*Table 7*). Stimulation 30/y produced significantly higher BF than the unstimulated control. No significant differences were detected between stimulations 30/y and 60/y and also between stimulation 4/y and the control.

Total solid content and dry rubber content. High tapping frequency of d/2 produced significantly lower TSC than low tapping frequency of d/4 (*Table 8*). Stimulations 30/y and 60/y led to significantly lower TSC when compared to stimulation 4/y or unstimulated control. Tapping and stimulation treatments affected DRC in a similar manner as TSC.

Physiological latex parameters associated with latex metabolism. Analyses of variance on physiological latex parameters associated with latex metabolism such as thiols content (R-SH) and Pi showed significant treatment effects while with sucrose and pH there was no significant treatment effects. No significant interaction effect was obtained for all the above parameters.

TABLE 5. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON PLUGGING INDEX

Tapping frequency	Stimulation frequency Control	4/y	30/y	60/y	Mean
½S d/2	3.93	2.47	2.14	1.55	2.52
½S d/4	4.55	4.09	2.37	1.86	3.22
Mean	4.24	3.28	2.26	1.71	2.87

$LSD_{0.05}$ (tapping frequency) = 0.38

$LSD_{0.05}$ (stimulation frequency) = 0.54

TABLE 6. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON INITIAL FLOW RATE (ML/MINUTE)

Tapping frequency	Stimulation frequency Control	 4/y	 30/y	 60/y	Mean
½S d/2	4.4	3.2	2.1	1.7	2.9
½S d/4	5.2	5.6	3.5	4.0	4.6
Mean	4.8	4.4	2.8	2.9	3.7

$LSD_{0.05}$ (tapping frequency) = 0.6

$LSD_{0.05}$ (stimulation frequency) = 0.8

TABLE 7. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON BOTTOM FRACTION (%)

Tapping frequency	Stimulation frequency Control	 4/y	 30/y	 60/y	Mean
½S d/2	21.9	21.1	25.6	25.1	23.4
½S d/4	14.5	17.1	20.6	18.7	17.7
Mean	18.2	19.1	23.1	21.9	20.6

$LSD_{0.05}$ (tapping frequency) = 2.5

$LSD_{0.05}$ (stimulation frequency) = 3.5

TABLE 8. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON TOTAL SOLID AND DRY RUBBER CONTENTS (%)

Tapping frequency	Stimulation frequency Control	 4/y	 30/y	 60/y	Mean
½S d/2	40.5 (35.1)	39.6 (35.0)	35.3 (31.2)	35.7 (32.0)	37.8 (33.3)
½S d/4	47.8 (41.6)	47.0 (40.2)	45.4 (40.1)	43.2 (38.5)	45.9 (40.1)
Mean	44.2 (38.4)	43.3 (37.6)	40.4 (35.7)	39.5 (35.3)	41.8 (36.7)

Figures in brackets are values for dry rubber contents

$LSD_{0.05}$ (tapping frequency) = 1.9 (1.5)

$LSD_{0.05}$ (stimulation frequency) = 2.7 (2.2)

Thiols content. High tapping frequency of d/2 produced significantly higher R-SH than low tapping frequency of d/4 (*Table 9*). No significant differences in R-SH were obtained between stimulated treatments and the unstimulated control.

Inorganic phosphorus. High tapping frequency of d/2 produced significantly higher Pi than lower tapping frequency d/4 (*Table 10*). Stimulation 60/y produced significantly higher Pi than stimulation 4/y or unstimulated control.

Sucrose. Tapping and stimulation treatments have no significant effect on the sucrose content of latex (*Table 11*).

pH. This parameter was also not affected by both tapping and stimulation treatments (*Table 12*).

DISCUSSION

There were no interaction effects between tapping and stimulation frequencies on mean dry rubber yield (g/t/t) during the first three years of tapping. Irrespective of tapping frequency, high stimulations (30/y and 60/y) tended to produce higher yield than low stimulation (4/y) or unstimulated control, though the magnitude of their yield differences declined with progressive years of tapping. The bulk of the increase in response to application of high dosages was only recorded in the first year of tapping, with increase thereafter being of a lower magnitude. During the fourth tapping year, no significant differences in yield were obtained among stimulated and unstimulated treatments under high tapping frequency of d/2 while under low tapping frequency d/4 the higher yields produced by high stimulations 30/y and 60/y were still above those of low stimulation 4/y or unstimulated control. However, yield responses did not increase proportionately with level of stimulation applied. The results suggest that high stimulations even though combined with low tapping frequency may result in low yield responses in later tapping years. In earlier trials it was shown that for sustained and positive response to be obtained over the long term, it would be preferable to apply low dosages of stimulant over a longer duration rather than high dosages over shorter duration[10].

The latex physiological parameters could be classified into two groups, namely a group which has a bearing on latex flow and another

TABLE 9. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON THIOLS CONTENT (mM)

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	0.41	0.38	0.35	0.39	0.38
½S d/4	0.34	0.31	0.33	0.34	0.33
Mean	0.38	0.35	0.34	0.37	0.36

$LSD_{0.05}$ (tapping frequency) = 0.03

TABLE 10. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON INORGANIC PHOSPHORUS (mM)

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	14.09	13.33	14.20	15.80	14.36
½S d/4	10.29	9.58	13.33	14.45	11.91
Mean	12.19	11.46	13.77	15.13	13.13

$LSD_{0.05}$ (tapping frequency) = 1.89

$LSD_{0.05}$ (stimulation frequency) = 2.68

TABLE 11. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON SUCROSE CONTENT (mM)

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	5.96	5.47	3.44	4.69	4.89
½S d/4	5.71	5.32	4.41	5.19	5.16
Mean	5.84	5.40	3.93	4.94	5.02

TABLE 12. EFFECTS OF TAPPING AND STIMULATION FREQUENCY ON LATEX pH

Tapping frequency	Stimulation frequency				Mean
	Control	4/y	30/y	60/y	
½S d/2	6.80	6.75	6.78	6.80	6.78
½S d/4	6.75	6.73	6.81	6.80	6.77
Mean	6.78	6.74	6.80	6.80	6.78

group on latex metabolism. The parameters in the former group include PI, IFR, DRC, TSC and BF. The latter group includes sugar, Pi, pH of latex and thiols (R-SH). It has been reported that some of these parameters such as TSC, DRC and thiols may have dual functions *i.e.* roles in both latex flow and metabolism[4,8]. The biological roles and significance of these parameters have been reported in various publications[4,5,8,11].

The values recorded for the various parameters would seem to indicate that the effects of intensive stimulation and higher tapping frequency on these parameters were similar. All parameters were significantly affected by both tapping and stimulation treatments except for sucrose and pH, while thiol content was only influenced by tapping treatment.

Trees tapped on d/4 frequency in contrast to d/2 frequency recorded consistently higher readings for the various latex physiological parameters associated with latex flow namely PI, IFR, TSC and DRC. This is likely to indicate that trees tapped on low frequency have longer time to regenerate the cell contents which results in higher values of TSC and DRC. Higher values of TSC and DRC, in turn, have an effect on latex viscosity[12] which could limit the latex flow[4,13]. This limitation on latex flow is also reflected by the high PI values recorded in these studies. The PI values for d/2-tapped trees being lower than d/4-tapped trees, would therefore be expected to give higher yields per tapping. However, this was not so in the present experiment. In relating the values of physiological latex parameters to yield performance, it must be pointed out that in the present experiment, yields were based on the means of four years of tapping whereas the physiological latex parameters were based on the means of eight months of recordings, towards the final stage of the yield recording period. Notwithstanding this disparity in periods of recording for yield and physiological latex parameters, the absence of a higher yield per tapping of d/2 tapped trees may be explained as such that, though a lower PI would have contributed to a higher volume of latex, nevertheless the dry rubber yields would be lower because of lower DRC values recorded with the higher frequency of tapping. Furthermore, the low initial flow rate for d/2 tapped trees could have resulted in the lower PI and yield per tapping. It is apparent from this study that different factors were limiting latex flow in d/2 and d/4 frequencies. Hence, in d/2 the limitation is the short intervals between tappings with inadequate replenishment, while in d/4 frequency it could be the high DRC and PI. Therefore, the application of stimulation to low frequency tapping is necessary to enhance latex flow and overcome flow limiting factors. Earlier works stressed that stimulation played a key role in achieving the desired productivity from low frequency tapping systems[14,15]. This was true in this study, since stimulation was effective in lowering the PI and DRC values with consequently better yields. This can be explained by the fact that stimulation delays plugging in stimulated trees and consequently increases the flow time resulting in yield increases[11,16].

Several researchers[17–20] have emphasised the role of lutoids in latex coagulation. It has been reported that the presence of high percentage of undamaged lutoids in the latex is a reflection of greater latex stability. The results obtained in this study on percentage of bottom fraction using the haematocrit technique showed significant differences among treatments. Trees tapped on d/2 frequency had higher volumes for bottom fraction than that of d/4 tapped trees. This could be explained by the fact that with low tapping frequency, there is higher turgor pressures within the vessels due to adequate regeneration of latex contents between tappings and loadings of sucrose in the vessels. Thus, when a tapping cut is opened in a low frequency tapped tree it can be expected that there will be a marked collapse in turgor pressure immediately under the cut with consequently far greater dilution effects[21]. The dilution effects could be attributed to movement of latex from the surrounding areas to the drainage area below the cut[22–25] and also movement of water and other minerals to this area, hence the far greater dilution effect on latex. The marked dilution effect can result in greater bottom fraction damage and consequently increased formation of plugs at points of destabilisation in the latex vessel. This could account for the reduced amount of bottom fraction recorded in low frequency tapped trees in this study.

Ethephon stimulated treatments, in this study, had more bottom fraction thus indicating greater stability of latex in stimulated trees. It is known that stimulation effect on latex flow is mediated through delayed plugging in the latex vessels[26]. The delayed plugging would mean less damaged lutoids with fewer plug formation. Thus, stimulation is most likely to confer greater stability on the lutoid particles with slower plugging and enhanced flow. Ethylene was reported to have a direct effect *in vitro* on the swelling of lutoid thus contributing to lesser number of damaged lutoids[27]. Therefore, the findings established in this study conform to expected trends previously reported. The reduced extent of damaged bottom fraction as reflected in higher values, as expected, resulted in better yield performance from stimulated treatments.

In relation to latex metabolism parameters, d/2 tapping and intensive stimulation (30/y and 60/y) resulted in higher value for Pi when compared to low frequency tapping (d/4) and unstimulated control or trees treated with mild stimulation (4/y). Inorganic phosphorus reflects the energy metabolism in laticiferous system[4]. It is formed *in situ* from the hydrolysis of phosphorylated molecules and that of inorganic pyrophosphate produced by the rubber transferase responsible for the lengthening of the polyisoprenic chain[28]. It influences to a considerable extent the various processes in the glucidic metabolism by providing necessary energy[29]. Therefore, the high content of free inorganic phosphorus recorded in d/2-tapped and intensively stimulated trees reflects the active metabolism in laticiferous cells of these trees. The higher metabolism observed in these trees is related to the higher yield produced by these trees and the consequent processes related to replenishment and regeneration in latex vessel of these trees.

Thiols are the main reducing molecules in latex which play an important role in protection of latex organelle membranes by neutralising the metabolic by-products of metabolic processes such as hydrogen peroxide (H_2O_2) and superoxide ions (O_2^-)[30]. The removal of free toxic oxygen radicals from the laticiferous system will contribute to latex stability and consequently increased duration of latex flow. Thiols have a role as potential activators of certain key enzymes in latex such as invertase and pyruvate kinase[31]. In this study, latex stimulation did not significantly affect thiol content but tapping frequency influenced the levels of thiol compounds in the latex. The higher values recorded in d/2 tapped trees may plausibly be explained by its dual functions in both latex metabolism and latex flow.

It has also been reported that stimulations resulted in lower sucrose content in latex vessel over the long-term[32–34]. It has been reported that there is an increasing sucrose content immediately at the first tapping after stimulation largely due to the 'sink effect' where sucrose was transported from the other parts of the rubber tree to the laticifers and from hydrolysis of starch reserves present in the bark[35]. In the present experiment, there were no significant differences in the sucrose contents among the various treatments. The lack of changes in the sucrose contents may be attributed to the decline in yield increases noted after the first tapping year, despite the intensity of stimulation.

The changes in latex physiological parameters obtained with intensive stimulation would indicate modifications in the laticiferous system. However, these changes have not resulted in development of physiological stress in the panel because there were no significant differences recorded in terms of dryness incidence among stimulation treatments, with the exception of trees stimulated with 60/y. The significant incidence of dryness in the latter treatment, however, was not accompanied with marked increase in yields because it was only marginally higher than that obtained with 30/y. The exhaustion theory with excessive drainage of assimilates resulting in dryness would not be applicable to these trees[36]. The other plausible explanation for higher incidence could be due to organelle damage from build up of toxic oxygen radicals in the laticiferous system with a reduction in thiol compounds. However, this was not noted in results obtained in this study. It is therefore unlikely that the higher incidence of dryness in the treatment with 60/y is a result of physiological stress in the panel. It could be due to other factors which are outside the scope of this study.

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Selection of Advanced Polycross Progenies in Hevea *Improvement*

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This paper reviews the progress made in the ortet (mother-tree) selection programme in Hevea *improvement carried out at the Rubber Research Institute of Malaysia. Since the 1970s, a concerted search for promising ortets from a population of 1.3 million seedlings derived from advanced polycross seed gardens* viz. *PBIG and PBFP trials, in the commercial plantations of Peninsular Malaysia has been made. Out of this sample, a total of 2613 genotypes (seedlings) has been selected for further test. Of the 1289 genotypes tested in the three field nurseries with two control clones PR 255 and RRIM 600, 34.1% showed mean yields which were equal or higher than the former while 59.7% showed equal or higher yields than the latter. In the first series of the selection, which was tested in large scale clone trials, a number of ortet materials showed yield levels higher than the control RRIM 600. Some of them had equal or higher mean yields than clones of the RRIM 800 series and promotion plot clones in adjacent fields. These results imply that selection of advanced polycross progenies (ortet selection) can be a useful complement to the normal hand (controlled) pollination programme in* Hevea *improvement.*

In the early days of rubber cultivation in the Far East at the end of the nineteenth century, the only planting material used was 'unselected seeds' from any suitable source[1]. The degree of selection practised was limited to the collection of seeds from areas of healthy, well grown trees which by the standards of the time were giving a high yield of latex.

Historically, Whitby was the first to report on the wide variability of productive capacity of the individual seedling trees[2,3]. His studies, started in 1913, showed that from a population of 1011 seedlings, 9.8% of the highest yielding seedlings produced 28% of the total crop.

It was only after the introduction of the brown budding technique in 1916 by Van Helten, Bodde and Tas that the full advantage was taken to vegetatively propagate outstanding mother-trees[2,4]. The first clone numbers to be released were Cramer's Cultuurtuin Ct 3 and Ct 9 resulting from the selection of 33 seedlings from Wickham trees from Penang to Java in Indonesia[5]. Subsequent selection from the progenies of Cramer's best mother-trees yielded other Ct clones including Ct 88. Mixed plantings of Ct 3, Ct 9 and Ct 88 later yielded well over 1700 kg per hectare[5]. The yield levels of these clones were very much higher than those of the unselected seedlings (496 kg/ha/yr)

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and the 'first mother-tree seedlings' (639 kg/ha/yr)[6].

Extensive mass (ortet) selection programmes were then carried out in areas where large population of mother-tree seedlings were available. Among the early primary clones disbudded were AVROS 33, AVROS 36, AVROS 49, AVROS 50, AVROS 52 and AVROS 80 in Sumatra; Tjir 1, Tjir 16, LCB 510 (PR 107), LCB 1320 and GT 1 in Java.

In the then Malaya, Sanderson and Sutcliffe of the Rubber Growers' Association selected and disbudded 21 highest yielding mother-trees from Pilmoor Estate in 1924. At about the same time, Major Gough the founder of Prang Besar Estate selected and disbudded 618 clones from a population of about one million seedlings in the Kajang district. The better known primary clones from these two selections were Pil A 44, Pil B 84, Pil B 16, PB 23, PB 25, PB 86, PB 186 and Gl 1[7,8].

The primary clones yielded two to three times that of the unselected seedlings. However, by the mid-1930s, breeders from both countries realised that mass selection for primary clones gave diminishing returns for their effort. Further progress would have to depend on the creation of improved populations for subsequent selection by intercrossing the primary clones. Hence, emphasis was shifted to controlled hand-pollination instead of mass selection[7].

While research efforts were mainly concentrated on controlled hand-pollination, seed gardens were later set up with improved clones as parents to produce high yielding polycross seeds as supplementary planting materials. The best known of these seedlings came from Prang Besar Isolation Garden/Gough Garden 1 (PBIG/GG 1), Gough Garden 2 (GG 2) and Prang Besar Further Proof (PBFP) trials. The seedlings derived from these seed gardens gave mean yields of 1200 kg per hectare per year. This was two to three times the yield of the old seedlings from which the first primary clones were selected. In consequence, the likelihood of finding high yielding clones from these new variable 'synthetic' populations is expected to be good.

In the early 1970s, the polycross seedling areas of PBIG/GG 1, GG 2 and PBFP trials found in commercial plantings totalled some 7700 hectares. These represent a population of more than two million mature seedling trees. This large population offers vast scope for selection. Faster results are also possible because all the seedlings were in mature stands and in tapping. Being in commercial plantings, there was no need to commit scarce experimental land nor funds for tapping. In consequence, the Rubber Research Institute of Malaysia (RRIM) in 1972 initiated a programme to systematically screen for outstanding individual seedlings from these advanced generation polycross materials. Early results of this work were most encouraging and subsequently the work was extended to cover seedling populations from PBIG/GG 4, GG 5 and GG 6. This paper reviews and updates the methodology and progress made in the mother-tree (ortet) selection programme carried out in the RRIM.

METHODOLOGY

Detailed description of the methodology has been covered by Ho[9] and Khoo[8]. Only a brief summary of the method is given here.

Field Selection in Estates

As the population for screening was vast and widespread, the co-operation and active participation of the estate staff was vital at the initial stages of screening to identify the high yielders. The tapper was given one month to mark out the 20 highest yielding trees in his task of about 500 trees. These selections were checked and confirmed by the tapping conductor and the trees were then numbered.

Monthly yield recording by cup coagulation of the selected trees on a normal tapping day was carried out for one year, resulting in 12 yield recordings for each tree. The cup-lumps were dried and weighed. Average individual tree yield was computed, ranked in descending order within each task and expressed as a percentage of the mean of the 20 trees within the task. This was done to minimise variations due to environmental factors such as age of trees, tapping panel, tapper's effect, tapping system, soil type and management system. Effectively, blocking was done on a task size basis.

The top two to three trees within a task were chosen for assessment of secondary characteristics such as branching habit, vigour, diseases, trunk condition, dryness and bark thickness.

Final selection for disbudding was based on the combined score of both yield and secondary characteristics which were arbitrarily assigned a weightage of 70% and 30%, respectively. Details of the scoring method can be found in Ho[7]. On the average, one tree was selected from each task of more than five hundred trees for further testing.

Nursery Selection

The selected mother-trees were disbudded into field nursery in RRIM Experiment Station (RRIES), Sungai Buloh at 122 cm × 122 cm spacing with six buddings per plot. The design was a randomised block with four replications. Two control clones, RRIM 600 and PR 255, were used.

Girth measurements were taken at 12 and 24 months from cut-back. The trees were test-tapped on S/2 d/2 100% after the second girth measurement. Three cycles of 15 tappings each were carried out. The trees were also scored for secondary characteristics. Final selection was based on the combined score of both yield (65%) and secondary characteristics (35%).

Small-scale Testing

Selections made from ortet nurseries were established alongside hand-pollinated progenies for further evaluation. These materials were planted at normal field spacing of small-scale clone trial. Plot size was eight trees in a row with two replications.

The trees were opened for tapping when at least 50% of them had reached 46 cm in girth. Tapping system was S/2 d/3 6/7 57%. Selections were made based on at least two-year yield records.

Large-scale Testing

From the nursery test-tapping results, a small number of the very best clones were selected for large-scale testing. This took the form of promotion plot clone trial and is similar to large-scale clone trials except that it has only two replications. The plot size was 56 trees (7 × 8) or 64 trees (8 × 8) and density was around 400 points per hectare.

When matured, yields of the plants were recorded based on S/2 d/2 6/7 86%. The usual method of monthly cup-coagulation to estimate yield was adopted.

RESULTS

Due to the large population available for selection not every field could be screened. Therefore the work had to be carried out in phases. There were altogether five phases. *Phase I* to *IV* covered the older seedling stands of mainly PBIG/GG 1 and GG 2 while *Phase V* covered PBIG/GG 4, 5 and 6.

Field Screening

Table 1 summarises all the five phases for easy comparison. *Phase I* covered 902 ha of about 280 000 seedlings in 17 estates. Most of the fields were PBIG/GG 1 and 2 with one field of PBFP and two fields of PBIG. A total of 10 580 seedlings was selected for one year of yield recording. From this yield result, 1387 mother-trees were short listed for scoring of secondary characters and 601 were finally disbudded for further evaluation in RRIES, Sungai Buloh.

Phase II screened through 805 ha from 32 fields in 21 estates. Approximately, a quarter of a million seedlings were involved from which 10 570 were yield recorded for one year and 1271 were short listed for scoring of secondary characters. The final number selected for disbudding was 592 ortets.

Phases III and *IV* also covered mainly PBIG/GG 1 and 2 and resulted in 295 and 597 ortets, respectively. Details of these two phases are also given in *Table 1*.

Phase V screening consisted of PBIG/GG 4, 5 and 6. The 24 fields in 21 estates covered 903 ha of 280 000 seedlings. The final selection for disbudding was 528 ortets.

The five phases of screening in estates went through 1.29 million seedlings in 4156 ha. A total of 2613 ortets was disbudded for further evaluation.

Nursery Test-tapping

Three phases of nursery evaluation have been completed. One of the other two nurseries is being test-tapped and the last one is being established.

Results of nursery test-tapping of materials from the first three phases are summarised in *Tables 2, 3* and *4*. In the first nursery, a total of 461 clones was evaluated *(Table 2)*. The test-tapping yields were grouped into five yield classes based on the trial mean yield and standard deviation. The distribution is slightly skewed to the left. The two control clones, PR 255 and RRIM 600, fell into the 'high' and 'above-average' classes, respectively. There were 47 clones in the 'high' class and 80 clones in the 'above-average' class. Of the ortet clones, 39 out-yielded control clone PR 255 while 107 out-yielded control clone RRIM 600.

The second nursery evaluated 425 clones and the distribution by yield classes is given in *Table 3*. The pattern followed that of the first nursery except that the mean yields were much higher because of good soil conditions in this nursery. The control clone, PR 255, is in the 'above-average' class and RRIM 600 is in the 'average' class. This means that a larger portion of the ortet population out-yielded the two control clones.

Results of test-tapping in the third nursery are summarised in *Table 4*. The pattern of distribution remained the same although the control clones PR 255 and RRIM 600 were reclassified as 'average' and 'below-average' class, respectively. Proportionately, a larger number of ortet clones in this nursery out-yielded the two control clones compared to the first two nurseries.

TABLE 1. PRELIMINARY SELECTION OF POLYCROSS SEEDLING POPULATION FROM COMMERCIAL ESTATES

Phase	Estate	Field	Area (ha)	Approximate population	Number of seedlings Selected for yield recording	Shortlisted for scoring	Selected for disbudding
I	17	30	902	280 000	10 580	1 387	601
II	21	32	805	250 000	10 570	1 271	592
III	7	16	579	180 000	6 520	764	295
IV	23	34	967	300 000	11 720	1 265	597
V	21	24	903	280 000	9 140	1 082	528
Total	89	136	4 156	1 290 000	48 530	5 769	2 613

TABLE 2. CLASSIFICATION OF CLONES BY MEAN YIELDS IN FIRST ORTET NURSERY

Yield class	Yield range (g/tree/cycle)	No. of clones	Yield of control (g/tree/cycle)
High	17.9 – 30.1	47	18.4 (PR 255)
Above average	14.7 – 17.8	80	15.2 (RRIM 600)
Average	10.3 – 14.6	179	
Below average	7.1 – 10.2	113	
Low	0.8 – 7.0	42	
Total		461	

Each cycle is a total of 15 tappings.

Comparing the results from the three nurseries, it is seen that proportionately larger numbers of ortet clones are out-yielding both the control clones as one progresses from the first nursery to the third. One possible reason is that the later populations are genetically superior to the earlier ones. Although the ortets may have come from the same PBIG/GG 1 and 2 seed-gardens, selective thinning was carried out in these seed-gardens in the early days to arrive at the best combination of parents. Another possible explanation is that the technique of selection improved with practice. The final outcome may actually be attributed to a combination of both these factors.

Small-scale Clone Trials

From the first nursery, 99 clones were selected and established in a small-scale clone trial. Unfortunately, extensive buffalo and cattle

TABLE 3. CLASSIFICATION OF CLONES BY MEAN YIELDS IN SECOND ORTET NURSERY

Yield class	Yield range (g/tree/cycle)	No. of clones	Yield of control (g/tree/cycle)
High	53.8 – 78.8	41	
Above average	45.1 – 53.7	77	48.1 (PR 255)
Average	33.5 – 45.0	160	40.3 (RRIM 600)
Below average	24.8 – 33.4	110	
Low	7.6 – 24.7	37	
Total		425	

Each cycle is a total of 15 tappings.

TABLE 4. CLASSIFICATION OF CLONES BY MEAN YIELDS IN THIRD ORTET NURSERY

Yield class	Yield range (g/tree/cycle)	No. of clones	Yield of control (g/tree/cycle)
High	39.7 – 62.0	45	
Above average	32.4 – 39.6	66	23.9 (PR 255)
Average	22.7 – 32.3	163	20.1 (RRIM 600)
Below average	15.4 – 22.6	90	
Low	4.9 – 15.3	39	
Total		403	

Each cycle is a total of 15 tappings.

damage to the trial made it impossible for meaningful recording and interpretation.

The second nursery produced 132 clones for testing in a small scale clone trial. Yield recording of these clones is in progress.

From the third nursery, only 37 clones were established in a small scale clone trial due to shortage of land. More land will be sought to test out the balance of 170 ortet clones.

Large-scale Clone Trials

Eleven clones from the first nursery were established in large scale clone trials in a randomised complete block design with two or three replications each.

Table 5 summarises the mean performance of the 11 ortet clones (OS 1–11) including RRIM 600 as control over a period of eight years of tapping in six locations in Peninsular Malaysia. In terms of yield/ha, the average yield of the 11 clones was 1262 kg/ha as compared to 1278 kg/ha of RRIM 600. Seven of the 11 ortet clones had recorded higher yield than the control clone. The higher yielding clones, namely OS 1, OS 3, OS 4 and OS 10 had out-yielded RRIM 600 by 17.5%, 8.0%, 6.8% and 6.7%, respectively.

In terms of yield/tree/tapping, the mean of the 11 ortet clones was 35.0 g/t/t which was slightly higher than the 32.2 g/t/t of the control RRIM 600. The higher yielding ortet clones were OS 2 (41.6 g/t/t), OS 5 (41.2 g/t/t), OS 7 (37.8 g/t/t), OS 1 (37.4 g/t/t) and OS 10 (37.3 g/t/t) which were 29.0%, 27.7%, 17.4%, 16.1% and 15.8% above the RRIM 600 control yield, respectively.

As for girth performance, all the 11 ortet clones showed early growth vigour. The average girths at fourth year after cut-back and at the time of tapping (G4, GO) were 37.8 cm and 48.5 cm, which were higher than the corresponding girths of 36.2 cm and 44.5 cm of the control. The girth increment on tapping and girth of closing *i.e.* after 8 years of tapping (GI, GC) for the ortet clones however showed a reverse trend. The average girth increment and girth at closing were 19.6 cm and 67.5 cm for the ortet clones as compared to 24.7 cm and 68.7 cm of the RRIM 600 control. The better girthing genotypes of the ortet clones *viz.* OS 2 (24.4 cm), OS 9 (22.7 cm), OS 1 (22.4 cm) and OS 11 (21.8 cm) had girth increment which ranged between 98.7% to 88.3% of the control clone (24.7 cm). The ortet clones which still maintained their good girth after 8 years of tapping occurred in OS 2 (74.5 cm), OS 6 (72.5 cm), OS 11 (71.1 cm), OS 1 (70.6 cm) and OS 9 (69.1 cm) which were higher than the control (68.7 cm).

The average virgin bark thickness (VB) at 6 years after tapping of the ortet clones (8.8 mm) was comparable to the control (8.7 mm). Six of the 11 ortet clones had slightly thicker virgin bark than the control. The average renewed bark thickness (RB) of the ortet clones (7.1 mm) was however poorer than the RRIM 600 control (7.6 mm). Only two ortet clones (OS 11 and OS 6) had slightly better renewed bark than the control.

The other secondary characteristics like wind damage incidence (WD) and dry tree incidence (BB) of the ortet clones were generally undesirable compared to the control clone.

The average wind damage incidence for the 11 ortet clones was 31.8 tree/ha as compared to 9.2 tree/ha of the control RRIM 600. Most of the clones had higher wind damage incidence than the control. Some ortet clones such as OS 2 (80.8 tree/ha), OS 7 (63.5 tree/ha), OS 3 (53.3 tree/ha), OS 8 (42.8 tree/ha) recorded 8.7–4.6 times the incidence of the control. OS 10 (7.4 tree/ha), OS 6 (8.3 tree/ha), OS 5 (10.3 tree/ha) had relatively low wind damage incidence to date.

The average dry tree incidence was 86.8 tree/ha for the 11 ortet clones, 1.5 times that of the control clone RRIM 600 (57.7 tree/ha). Clones OS 4 (107.8 tree/ha), OS 7 (104.9 tree/ha), OS 10 (101.2 tree/ha), OS 2 (97.4 tree/ha) were among the ortet clones having higher dry tree incidence recorded. Relatively lower dry tree incidence was recorded in OS 9 (52.5 tree/ha), OS 1 (62.7 tree/ha), OS 11 (63.1 tree/ha) and OS 6 (87.7 tree/ha).

TABLE 5. YIELD AND SOME SECONDARY CHARACTERISTICS OF CLONES FROM ORTET SELECTION

Clones	MY 8 (kg/ha)	MY 8 (g/t/t)	G4 (cm)	GO (cm)	GC (cm)	GI (cm)	VB (mm)	RB (mm)	WD (tree/ha)	BB (tree/ha)
OS 1	1 502	37.4	37.9	48.8	70.6	22.4	9.0	7.2	19.5	62.7
OS 3	1 380	36.7	37.2	48.5	67.8	20.8	9.1	7.4	53.3	92.3
OS 4	1 365	35.3	37.8	46.5	59.6	12.3	7.4	5.7	29.5	107.8
OS 10	1 364	37.3	36.8	47.6	64.8	18.3	8.7	6.9	7.4	101.2
OS 5	1 355	41.2	35.6	48.8	64.6	18.8	9.1	7.4	10.3	89.6
OS 7	1 316	37.8	39.3	48.7	63.7	15.0	8.8	7.0	63.5	104.9
OS 2	1 287	41.6	37.2	50.0	74.5	24.4	8.7	7.3	80.8	97.4
OS 11	1 125	27.2	39.0	50.1	71.1	21.8	9.9	8.2	11.4	63.1
OS 8	1 119	37.3	37.5	48.1	64.2	17.4	8.2	6.9	42.8	96.1
OS 6	1 069	27.7	39.2	50.3	72.5	21.7	9.6	7.9	8.3	87.7
OS 9	1 000	25.3	37.7	46.5	69.1	22.7	7.9	6.8	23.0	52.5
RRIM 600 (Control)	1 278	32.2	36.2	44.5	68.7	24.7	8.7	7.6	9.2	57.7

Mean over 6 trials

MY : Mean yield
G4 : Girth at 4th year
GO : Girth at opening
GC : Girth after 8 years of tapping
GI : Girth increment over 8 years of tapping
VB : Virgin bark thickness at 6th year of tapping
RB : Renewed bark thickness at 6th year of tapping
WD : Wind damage incidence
BB : Dry tree incidence

Table 6 compares selections from ortet selection and from hand pollination programme, *i.e.* RRIM 800 series and PB 300 series with reference to yield performance of three trials tested in adjoining sites. Generally, the ortets, RRIM 800 series and PB 300 series clones tested yielded slightly better than the control RRIM 600 in terms of yield/ha/year and yield/tree/tapping. A few of the better selections from ortet and hand pollination programmes out-yielded the control by up to 29% (OS 1 and PB 330) and 43% (RRIM 805) in terms of yield/ha/year. In terms of yield/tree/tapping, OS 2, PB 311 and RRIM 809 out-yielded the control by between 31.3% and 45.5%. In addition, some of the better yielding OS clones showed comparable yield potential with hand pollinated selections though in some cases, the better yielding hand pollination selections may have slightly higher absolute yield.

The OS clones resembled the RRIM 800 and PB 300 series selections in having early immature vigour (mean for GO of OS clones: 46.8 cm; RRIM 800 series clones: 47.0 cm; PB 300 series clones: 47.9 cm), below average

TABLE 6. MEAN YIELD OF ORTET, RRIM 800 SERIES AND PB 300 SERIES CLONES OVER 8 YEARS OF TAPPING TESTED IN 3 LOCATIONS

Genotype	Mean yield		Better yielding clones	
	kg/ha	g/t/t	kg/ha	g/t/t
Ortet				
OS1–11	1 195	36.2	OS 1 [1 515][b]	OS 2 [43.2]
	(932–1 515)[a]	(26.1–43.2)	OS 3 [1 300]	OS 5 [42.6]
			OS 10 [1 266]	OS 8 [41.0]
			OS 4 [1 259]	OS 7 [40.5]
RRIM 800 series				
RRIM 805 – 810	1 397	39.0	RRIM 805 [1 677]	RRIM 809 [47.9]
	(1 201–1 677)	(32.7–40.5)	RRIM 806 [1 481]	RRIM 806 [40.5]
			RRIM 810 [1 369]	RRIM 805 [40.2]
PB 300 series				
PB 310, PB 311,	1 198	36.0	PB 330 [1 519]	PB 311 [47.0]
PB 312, PB 324	(955–1 519)	(27.7–47.0)	PB 310 [1 296]	PB 310 [35.2]
PB 330			PB 324 [1 126]	PB 312 [35.2]
				PB 330 [35.0]
Control				
RRIM 600	1 173	32.9	RRIM 600 [1 173]	RRIM 600 [32.9]

()[a]: Figure within bracket refers to the lowest and highest yield of the selections

[][b]: Figure within bracket refers to the mean yield of corresponding clone

girth rate (mean for GI of OS clones: 19.6 cm; RRIM 800 series clones: 21.4 cm; PB 300 series clones: 25.8 cm; compared to RRIM 600: 30.6 cm), higher incidence of wind damage (mean of OS clones: 50.0 tree/ha with range 11.5–105.4 tree/ha; RRIM 800 series clones: 21.6 tree/ha with range 9.0–53.7 tree/ha; PB 300 series clones: 56.4 tree/ha with range 20.8–150.7 tree/ha compared to RRIM 600: 14.3 tree/ha) and dry tree incidence (mean of OS clones: 70.0 tree/ha with range 39.8–94.8 tree/ha; RRIM 800 series clones: 70.5 tree/ha with range 42.2–106.6 tree/ha; PB 300 series clones: 56.4 tree/ha with range 30.4–81.6 tree/ha compared to RRIM 600: 37.0 tree/ha).

In one of the six ortet trials where 10 promotion plot clones were tested in adjacent site, the best yielding ortet selection (OS 1: 1681 kg/ha; 46.2 g/t/t) was also comparable to the promising PC selections from the more recent hand-pollination programme (PC 92: 1779 kg/ha, 67.5 g/t/t; PC 71: 1548 kg/ha, 53.3 g/t/t).

DISCUSSION AND CONCLUSION

The method of ortet selection described is at best expedient. Prime considerations were constraints of fund, manpower and time. The approach adopted was aimed at extensive rather than intensive coverage. A total of 1.29 million seedlings were screened rather rapidly with minimal cost and manpower.

In view of the fact that hand-pollination programmes have problems with low fruit set (ranging from 0% to 5%), the advanced generation polycross seedlings are considered as an attractive supplement to the main breeding (hand pollination) programme.

Test-tapping results of the nurseries appear promising. Admittedly, early test-tapping is not a refined yield predictor but its use for separating groups of good clones from poorer ones is reliable. As mentioned earlier, each subsequent nursery gave higher proportion of high yielding clones. This could be due to better genetic materials or more efficient selection skills or probably a combination of both.

Results of large-scale testing of ortet clones over eight years of tapping are promising. The secondary characteristics, with the exception of immature growth vigour are generally inferior to those of the control clone RRIM 600. However, yield for the first 8 years shows that the ortet clones, in general, were better yielding than RRIM 600. When compared as a group to the RRIM 800 series (second selection) clones and the Promotion clones in corresponding trials, the ortet clones appear favourable. Their yield levels were just as good, if not better, than the best ones from the hand-pollination programme. This suggests that the ortet programme can be a very useful supplementary source of improved genetic materials for further evaluation.

Other attractive features of the ortet programme is the sheer size of its population of at least several million seedlings. No additional experimental land need to be committed and routine tapping is in progress permitting easy first round selection. More such fields of advanced generation polycross seedlings will be available in the future. The mean yield of these advance generation polycross seedlings is high. The possible parents are known to be good for breeding purposes.

Based on all the above considerations, ortet selection programme seems to be heading in the right direction. Hopefully in the not too distant future, some high yielding ortet clones may find their way into the Planting Recommendations.

It should be mentioned that at this juncture, the selected ortet clones, such as OS 1, OS 2, OS 5 and OS 3, although shown to be higher yielding than RRIM 600 control, unfortunately had some secondary defects. Unfavourable characters such as poorer girthing rate on tapping, higher wind damage and dry tree incidence were found to be associated with the selected higher yielding materials. In consequence, these OS clones have a number of limitations which would not permit them to be accepted as planting materials for wide-scale planting.

In view of all this, it is perhaps important that more advanced generation seedgardens be set up in the future. In addition to providing direct planting materials, seedling stands derived from these seedgardens will form the population for future ortet work. As commercial planting of rubber is geared towards a few high yielding clones, these seedling populations represent a good reservoir of genetic variability that is so vital to preserve.

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