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Contents

J. nat. Rubb. Res.
Volume 7(2), 1992

THE CHEMISTRY OF THE LATEX PREVULCANISATION PROCESS. PART 1. MIGRATION OF REACTANTS FROM SOLID PHASE INTO RUBBER PARTICLES 85
M. Porter, Rosemaze Rawi and Sapi'ai Abdul Rahim

PROTEINS OF NATURAL RUBBER LATEX CONCENTRATE 102
H. Hasma

STRESS RELAXATION IN NATURAL RUBBER BALE CRYSTALLISATION DURING STORAGE AT LOW TEMPERATURES 113
P. Benoist, R. Chasset and H. de Livonniere

A 100-YEAR CASE STUDY OF THE LIFE PREDICTION OF NATURAL RUBBER FOR AN ENGINEERING APPLICATION 126
Kamarudin Ab. Malek and A. Stevenson

LEARNING-BY-DOING IN PRODUCTION FUNCTION: THE CASE OF THE MALAYSIAN RUBBER ESTATE SECTOR 142
Muzafar Shah Habibullah

SHORT COMMUNICATION: FTIR STUDIES ON AMINO GROUPS IN PURIFIED *HEVEA* RUBBER 152
Eng Aik-Hwee, Yasuyuki Tanaka and Gan Seng-Neon

J. nat. Rubb. Res., 7(2), 85 – 101

Chemistry of the Latex Prevulcanisation Process. Part 1. Migration of Reactants from the Solid Phase into Rubber Particles

M. PORTER*, ROSEMAZE RAWI* AND SAPI'AI ABDUL RAHIM*

High-ammonia natural rubber latex has been prevulcanised with a zinc diethyldithiocarbamate-accelerated sulphur system in which either the sulphur or the accelerator was incorporated into the latex from a solid deposit on the wall of the containing vessel. The occurrence of prevulcanisation under these conditions is best explained on the thesis that both the accelerator and sulphur dissolve in the aqueous serum of the latex before migrating into the rubber phase. Heating the uncompounded latex in a sulphur-coated flask and subsequently compounding it with accelerator in a separate vessel and then heating it, also causes prevulcanisation, demonstrating that the presence of other ingredients is not necessary for sulphur to dissolve in the latex. However, the degree of prevulcanisation is limited by the quantity of sulphur dissolved. Similarly, latex can be prevulcanised by heating it in a ZDEC-coated flask (in the presence of diethylamine), compounding it with a sulphur dispersion in a separate step, and reheating it. In this case, the degree of prevulcanisation is not limited, presumably because ZDEC is continuously regenerated as crosslinking proceeds.

Aqueous leaching of films deposited from a prevulcanised latex usually causes increases in film modulus and, particularly, strength. In the experiments described, aqueous leaching had very little effect on these or other stress-strain properties.

Prevulcanisation of a rubber latex may be defined as a process in which crosslinking of the rubber takes place inside discrete particles dispersed in the aqueous serum of the latex. Subsequent formation of a coherent film of vulcanised rubber by deposition and drying should not, according to this definition, involve further crosslinking of the rubber. In practice, unless the vulcanising ingredients have been exhausted or removed, this condition is not met. In many cases, manufacturers rely on further crosslinking taking place in the dry, or drying, film (post vulcanisation) to achieve the product properties they require.

The process of prevulcanisation of natural rubber (NR) latex has been known for more than seventy years[1] but many aspects of it are not at all well understood. These include:

- The means by which the vulcanisation chemicals, originally present as dispersed solids, are transported into the rubber particles
- The extent to which the chemistry of crosslinking resembles that which is believed to occur during the vulcanisation of bulk rubber
- The structure of the wet gel deposit and the factors which affect its cohesion
- The structure of the dry film and the factors responsible for its physical properties, especially its strength

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- The physical chemistry of the changes brought about by leaching the wet gel or dry film in water.

This series of papers is concerned only with the first two of these aspects: the organic and physical chemistry of the vulcanisation process *per se*.

Present Understanding

Transport through the aqueous phase. Blackley[2] recently discussed the nature of the chemical processes which must lead from the solid reactants dispersed in the aqueous phase to the sulphur crosslinking of rubber molecules inside colloidal particles. The three types of reactant: sulphur, zinc oxide and (usually) a zinc dialkyldithiocarbamate, are essentially insoluble in water and some commentators on the process have postulated that reaction takes place as a result of direct contact between particles of reactant and rubber[3,4] or have apparently accepted this as the mechanism[5,6]. Others have started from the premise that the reactants must dissolve in the aqueous phase before diffusing into the rubber particles[2, 7-10]. What has always remained unclear is the form in which this dissolution occurs, whether the reactants undergo any prior reaction in the aqueous phase, and the nature of the diffusing species.

It has been known for many years that zinc oxide is soluble in ammoniated latex[11]. Dissolution depends upon the presence in the latex of acidic substances which form ammonium ions from the ammonia and promote the formation of soluble, positively charged, zinc ammine complexes. These complexes are transported at least as far as the surface of the rubber particles[11]. Hence a well-established mechanism for the transport of zinc ions from zinc oxide to the rubber particles exists. However, this appears to be irrelevant so far as the chemistry of prevulcanisation is concerned since the presence of zinc oxide, although beneficial, is not necessary for prevulcanisation to take place, provided the accelerator being used contains zinc[12]. As in the case of bulk rubber vulcanisation, the function of zinc oxide in the vulcanisation process appears to be to act largely as a secondary source of zinc, to regenerate the accelerator as it becomes consumed[2]. Under the chemically most simple conditions then – those in which sulphur and a zinc dialkyldithiocarbamate are used – only two vulcanising reagents have to be considered.

The transfer of sulphur from its solid dispersion into the latex rubber particles is clearly a rapid process: with a very active accelerator, such as zinc dibutyldithiocarbamate (ZDBC), detectable crosslinking[13] of the rubber occurs in less than 12 h at 27°C. Although milling sulphur with a surfactant in water to provide a fine dispersion is a ubiquitous practice, it is known to be unnecessary for vulcanisation to take place[9,14]. More recently, Gorton and Pendle[15] showed that variation of the average particle size of the sulphur dispersion over quite a wide range (number-average particle diameter, 1.5-13 μm) has no effect on the rate or extent of crosslinking. This finding indicated that the sulphur either dissolves in the aqueous serum or, possibly, that it reacts with the accelerator (perhaps in the presence of the natural substances in the serum) to form some sort of colloidal species which is transported to the rubber particle surface. The latter possibility was suggested by the observation[2] that the initial rate of crosslinking of a synthetic polyisoprene latex was proportional to the number of particles present and not to their total surface area, which would have been expected had the reagents been in solution. Transport of sulphur through the serum by one of these routes had already been demonstrated by Van Dalfsen[7], who regarded the process as one of dissolution of sulphur in the serum.

In fact, the presence of the accelerator is known not to be necessary for the sulphur to be transported into the rubber particles. Although sulphur is said not to be appreciably soluble in aqueous ammonia[9,16], it is soluble in the serum of ammoniated latex. This was most convincingly shown by Van Gils[9] who also demonstrated that sulphur is soluble in a solution of cysteine (actually the hydrochloride) in water. Cysteine is present in natural latex[17] and it was inferred that other amino acids and/or proteins present in latex might behave likewise. The activity of such substances in prevulcanisation has recently been demonstrated[18] using a deproteinised and purified latex. In a zinc diethyldithiocarbamate(ZDEC)-accelerated system at 60°C, the crosslinking of this latex showed an induction period and was much slower than that of a standard high-ammonia (HA) latex. Addition of ammonia to the purified latex had no effect but addition of the amino acid, 3-aminopropionic acid (1% on the latex) accelerated crosslinking strongly (as did addition of aqueous serum from HA latex). However, it must be pointed out that, while the presence of such substances accelerates the prevulcanisation process, their absence does not actually prevent prevulcanisation from occurring[2]. In these cases, some other species would have to promote the dissolution of sulphur in the aqueous phase.

The behaviour of a zinc dialkyldithiocarbamate is different. The rate of its disappearance during prevulcanisation appears to follow zero-order kinetics[2,10,19]. This indicates either: i) that its concentration in the aqueous serum is constant, the bulk of the solid remaining undissolved[2,19]; or ii) that its disappearance is limited by the rate of diffusion of the accelerator (or a reaction intermediate derived from it) into the rubber[20,21]. The first of these explanations is more likely to be correct since the zero-order rate of disappearance of the accelerator varies with the initial accelerator concentration (or with the accelerator:sulphur ratio)[2,10,19]; a diffusion-controlled process might be expected to be independent of this external factor. Loh[10] found the solubility of ZDBC in water and in ammoniated water (pH 10.2) to be very small (0.003% – 0.005%). In diluted serum from NR latex, it was appreciably higher (0.011%). However, it appears that interaction between sulphur and the zinc dithiocarbamate may take place in the aqueous phase to form a species which is more soluble than the dithiocarbamate in both water and rubber. This was deduced by Blackley[2] on the basis of opacity or clarity of films containing one or both components and is supported by the fact that an increase in initial sulphur concentration raises the rate of disappearance of ZDBC. However, it should be pointed out that any deduction made on the basis of the disappearance of a zinc dithiocarbamate may be vitiated by the fact that a dithiocarbamate can disappear from ammoniated latex by a reaction unrelated to vulcanisation. Thus, Loh[10] observed a significant zero-order loss of ZDBC even in the absence of sulphur, while Loadman[22] noted its reaction with some unextractable component of NR. This did not occur with a synthetic polyisoprene latex. These observations are likely to be connected with the better documented decomposition of zinc dimethyldithiocarbamate (ZDMC) in latex[23].

There is, nevertheless, further evidence that dissolution of the accelerator in the aqueous phase is a rate-limiting feature: the rate and extent of crosslinking were hardly affected by changes in the initial dithiocarbamate concentration at constant sulphur concentration[20,21], or by dithiocarbamate concentrations above 1 p.h.r. when the sulphur concentration was changed *pro rata*[20].

In summary, present evidence favours the concept that both sulphur and zinc dithiocarbamate accelerator dissolve in some form in the aqueous serum of the latex and are thus transported to the surface of the rubber particles from where they diffuse into the rubber and crosslink it. Sulphur appears to undergo this process relatively rapidly, dissolving in the latex serum without the aid of the accelerator. In contrast, the low solubility of the accelerator in the serum appears to be rate-limiting but its solubility seems to be increased by the presence of sulphur, indicating that some degree of interaction may take place in the aqueous phase and before the reactants enter the rubber phase.

Reaction in the rubber particles. The sequence of events following the arrival of the vulcanising reagents at the surface of the rubber particles is also of some importance. The main question, of course, concerns the relative rates of diffusion of the reagents in the rubber and of their reaction with the rubber to form crosslinks. For one extreme (diffusion much faster than reaction), one can visualise homogeneously crosslinked rubber particles, irrespective of size; for the other (reaction much faster than diffusion), one would envisage the production, from large particles at least, of a highly crosslinked shell of rubber surrounding an uncrosslinked core. These two scenarios would be expected to be highly important for film formation from the vulcanised latex. If the particles are preferentially crosslinked near their surfaces, the reduced mobility of the rubber chains there should make it more difficult for the particles to coalesce and a highly coherent film would not be expected. In contrast, a latex composed of homogeneously crosslinked particles might be expected to coalesce well and to form a film with optimal physical properties.

At present, there seems to be no direct evidence which bears on this point. Hu and co-workers[24] claim, without providing evidence, that the rate of crosslinking is much greater than the rate of diffusion. They deduce that crosslinking occurs rapidly as the vulcanising agents enter the surface of the rubber particles and that the crosslinks formed hinder further diffusion of the reagents into the interior of the particles. Support for this conclusion is elicited from the work of Grushetskaya *et al.*[25] but this, and their associated work[26-29], all appear to apply to crosslinking *after* a film has been formed (although it is difficult to be certain in all cases because Russian authors seldom provide complete experimental details of their work). Hu and co-workers[24] also hold the view that the rubber molecules in latex particles are considerably more mobile than those in bulk rubber and that, when crosslinked with 'flexible' polysulphide crosslinks, it is possible for the crosslinks originally formed near the surface to become gradually re-distributed throughout the particle. The proposed time scale for this process is not at all clear. However, the rates of crosslinking exhibited by the various dithiocarbamate accelerators vary over a wide range and if crosslinking were so much faster than diffusion that surface crosslinking was prevalent, one might expect to see evidence of poor film properties from a ZDBC-accelerated prevulcanised latex at 50°C compared, for example, to those from a ZDMC-accelerated prevulcanised latex prepared at 70°C, after extended maturation at room temperature. The question of homogeneity of crosslinking inside the particles in a prevulcanised latex must be regarded as an open one.

This paper is concerned with the processes by which sulphur and a zinc dithiocarbamate are each able to migrate from a separate crystalline phase to the surface of rubber particles in a HA latex. The starting point for this work was the fundamental experiments of Van Gils[9] who showed *inter alia* that sulphur could transfer from solid lumps in a muslin bag or from a deposit on the wall of a container into the rubber particles of a latex, thus demonstrating the solubility of sulphur in latex serum. This approach has here been applied also to a zinc dithiocarbamate accelerator and has been extended to demonstrating that crosslinking of the rubber can also ensue.

EXPERIMENTAL

Latex Compounding and Vulcanisation

HA latex was a commercial sample, preserved with a 0.7 wt.% ammonia. (Found: N, 0.27%; ash, 0.46%; on evaporated film.) All compounding ingredients were commercial chemicals, used without further purification. Aqueous dispersions of sulphur, zinc oxide and ZDEC were prepared by ball milling under standard conditions[30].

Latex was compounded according to one of the formulations shown in *Table 1*. After compounding, the mixture was stirred slowly for 0.5 h and allowed to stand for 2 h before use. In some cases, longer maturation times were used. Where relevant, these are indicated in the tables and figures.

In experiments where sulphur was incorporated into the latex from a solid deposit on the wall of a flask, sulphur was melted in the lower section of a flanged flask and the flask rotated until the interior surface was coated and then allowed to cool. To coat a flask with ZDEC, it was found to be preferable to melt ZDEC carefully in a separate vessel and pour it into a flanged flask with simultaneous rotation of the flask to distribute the material over the interior surface.

Incubation (heating an uncomponded or incompletely compounded latex in a flask coated with a compounding ingredient) and prevulcanisation experiments were carried out in the covered flask immersed in a water bath maintained at 70°C. The latex was subjected to continuous slow stirring and samples were withdrawn at intervals for chloroform number testing and for preparing evaporated films, *ca.* 0.8 mm thick, by casting on level glass plates. After 24 h, the films were removed from the plates, hung in air and, when translucent, were placed in a vacuum desiccator over silica gel. They were removed from the desiccator and acclimatised in the test laboratory before tensile testing, which was carried out according to *ISO 37*.

Where leaching was carried out, the dried cast film was immersed in a large volume of de-ionised water for 16 h at room temperature (*ca.* 28°C) and then dried and tested as before.

In considering the data in the tables, it should be borne in mind that chloroform number is an instantaneous test which provides a measure of the extent of cross-linking at the time the test was made, whereas the physical measurements were made on films some five to ten days after casting. While ZDEC is a relatively slow accelerator at the ambient temperature of about 28°C, some degree of post-cure may take place between casting and measuring, and this will be reflected in apparently higher physical properties than would be indicated by the chloroform number.

Equilibrium Volume Swelling Measurements

Accurately weighed samples (*ca.* 0.2 g) cut from the vulcanised films (unleached except where indicated otherwise) were immersed in toluene at 35°C or 40°C and allowed to swell for 48 h (with one change

TABLE 1. PREVULCANISATION FORMULATIONS

Ingredient	Quantity (parts by dry weight) of mix				
	A	B	C	D	E
60% HA latex	100.0	100.0	100.0	100.0	100.0
10% Potassium hydroxide	0.3	0.3	0.3	0.3	–
50% Zinc oxide dispersion	0.25	0.25	0.25	0.25	0.25
50% Sulphur dispersion	–	–	1.5	1.5	1.5
50% ZDEC dispersion	1.5	1.5	–	–	–
10% Diethylamine solution	–	2.0	–	2.0	–

TABLE 2. PREVULCANISATION AT 70°C OF HA LATEX, COMPOUNDED ACCORDING TO MIX A, IN A FLASK COATED WITH SOLID SULPHUR

PrVn time (h)	Chloroform number	M300 (MPa) UL	M300 (MPa) L	TS (MPa) UL	TS (MPa) L	EB (%) UL	EB (%) L	V_r at 40°C in toluene UL
0	1	0.52	0.57	1.9	2.7	844	843	0.031
2	1	0.52	0.58	2.7	3.7	863	970	0.0345
5	2	0.53	0.58	5.2	5.2	976	1000	0.047
9	2	0.57	0.63	7.1	7.3	966	989	0.068
14	3	0.69	0.69	13.0	13.9	970	959	0.079
24	4	0.78	0.80	17.8	19.4	970	953	0.113

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

of solvent after 24 h). At the end of this period, surface solvent was removed with filter paper and the sample weighed in a closed weighing bottle. The imbibed toluene was removed by heating at 60°C in an air oven (to constant weight). Values of V_r, the volume fraction of rubber in the swollen gel, were calculated and corrected as previously described[31,32].

RESULTS

Incorporation of Sulphur from a Solid Deposit

The results presented in *Tables 2* and *3* and *Figure 1* show clearly that it is possible to prevulcanise NR latex utilising sulphur from a solid deposit on the wall of the containing vessel. Reaction was slow, the

TABLE 3. PREVULCANISATION AT 70°C OF HA LATEX, COMPOUNDED ACCORDING TO MIX B, IN A FLASK COATED WITH SOLID SULPHUR

PrVn time (h)	Chloroform number	M300 (MPa) UL	M300 (MPa) L	TS (MPa) UL	TS (MPa) L	EB (%) UL	EB (%) L	V_r at 40°C in toluene UL
0	1	0.50	0.55	2.1	3.1	932	892	0.024
2	1	0.50	0.58	4.6	5.0	990	992	–
5	2	0.58	0.64	7.7	9.0	900	967	0.062
9	3	0.70	0.74	13.0	13.3	924	913	0.092
14	3	0.81	0.88	17.3	20.9	811	827	0.113
24	3	0.96	1.15	18.2	28.9	809	737	0.145

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

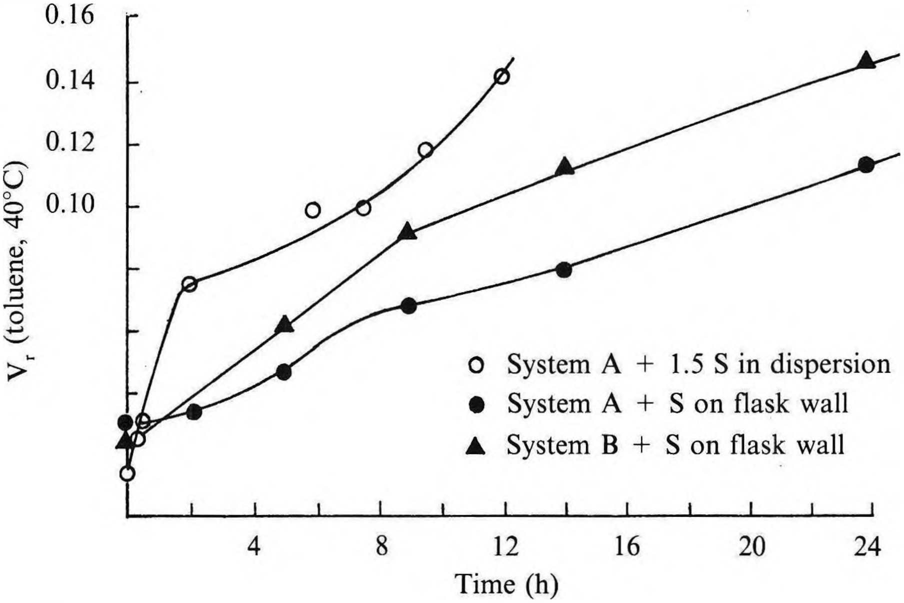

Figure 1. Influence of the form of sulphur on the development of crosslinking (as measured by equilibrium volume swelling) during prevulcanisation with a ZDEC-accelerated system at 70°C.

chloroform number still not having reached 4 in 14 h at 70°C. Nevertheless, M300 values (the stress values at 300% extension) of 0.8 MPa and tensile strength (TS) values of nearly 20 MPa indicate that considerable vulcanisation had occurred after 24 h. This was confirmed by an equilibrium V_r value in toluene of 0.113. For comparison, vulcanisation of *System A* (*Table 1*) containing 1.5 p.h.r. sulphur as a ball-milled dispersion, in addition to the other ingredients, produced, after 2 h at 70°C, the following values (for leached film)[33]: M300, 0.96 MPa; TS, 28.6 MPa. The rate of change of V_r with time is compared with that for the same system[13] in *Figure 1*. (In preparing the deposit, care was taken not to heat the sulphur further above its melting point than was necessary to achieve adequate flow. Nevertheless, allotropic transformation cannot be excluded.)

The inclusion of diethylamine in the latex formulation (*Mix B*) increased the rate of vulcanisation, but only by a factor of about two (*Table 3*) and most of its effect was seen in the early stages of the reaction (*Figure 1*). Primary and secondary amines are known to facilitate the dissolution of sulphur[34,35] and have been recommended[36,37] for activation of sulphur in latex formulations. Whether diethylamine was present or not, prevulcanisation did not show any sign of slowing down in 24 h at 70°C. At the end of this period, the sulphur on the wall of the flask appeared to be heavily coated with coagulum; this evidently did not prevent further reaction.

To determine whether the presence of other compounding ingredients was necessary to effect dissolution of the sulphur, HA latex was heated for a 24-h period at 70°C in a sulphur-coated flask and then transferred to a second flask, where it was compounded with solid dispersions according to *Mix A*, and allowed to stand (mature) for 2 h. As expected, there was little evidence for any crosslinking of the rubber phase having occurred at this point but on further heating at 70°C, a limited amount of crosslinking took place in a 3-h period, after which extensive coagulation occurred (*Table 4*).

However, even after 3 h, there was already evidence that the degree of prevulcanisation was reaching a plateau well short of a level where the best properties would be expected. Thus, the chloroform number did not reach 4 and values of 0.5 MPa for M300 and 10 MPa for TS for cast films were not exceeded. It appeared that prevulcanisation was occurring but to only a limited extent, which was presumably controlled by the amount of sulphur which had dissolved into the latex during the first heating period. It seems that, while sulphur can dissolve in the serum of HA latex, the rate of dissolution is very slow in the absence of ancillary chemicals. This accords with Van Gils's observations, which were made at room temperature[9].

Addition of diethylamine to the latex before incubation in the sulphur-coated flask did not significantly affect the results (*Table 5*). The absence of an effect indicates that the modest acceleration of the earlier prevulcanisation (*Figure 1*) may be due to interaction of the diethylamine with the dithiocarbamate component.

Retaining the 24-h initial heating time but increasing the maturation period at room temperature after subsequent compounding gave a more positive result on prevulcanisation (*Table 6*). A chloroform number of 4 was reached in 3 h at 70°C, after which time the TS of a cast film was 17 MPa. Evidence from TS (*Figure 2*) and equilibrium swelling values (*Figure 3*) indicates that prevulcanisation was rapid for 1–1.5 h and then it became much slower and a plateau was reached, suggesting again that the quantity of sulphur taken up was a limiting feature. However, the results of *Tables 4* and *6*, and *Figures 2* and *3* point to the limiting crosslink density being higher after 17 h maturation than after 2 h maturation. Since the quantity of sulphur in the system is already fixed by the incubation period, this observation implies either that there is a re-distribution of sulphur between the aqueous and polymer phases during the maturation period or that longer maturation allows more accelerator or accelerator complex to enter the rubber phase, producing more efficient crosslinking from the sulphur already present there.

TABLE 4. PREVULCANISATION AT 70°C OF HA LATEX WHICH HAD BEEN HEATED IN A SULPHUR-COATED FLASK FOR 24 H AT 70°C, COOLED, COMPOUNDED ACCORDING TO MIX A AND MATURED FOR 2 H AT ROOM TEMPERATURE

PrVn time (min)	Chloroform number	M300 (MPa)		TS (MPa)		EB (%)		V_r at 40°C in toluene
		UL	L	UL	L	UL	L	UL
0	1	0.25	0.20	4.3	2.6	879	849	0.031
10	1	0.26	0.24	5.4	3.1	978	970	–
30	2	0.33	0.26	5.7	4.2	998	988	0.048
60	2	0.34	0.36	7.5	5.9	1 006	999	0.062
90	3	0.37	0.37	8.1	7.1	1 010	995	0.062
120	3	0.37	0.48	8.6	7.6	990	995	0.064
180	3	0.39	0.50	9.2	9.8	985	989	0.065

PrVn = Prevulcanisation

EB = Elongation at break

UL = Unleached; L = Leached

TABLE 5. PREVULCANISATION AT 70°C OF HA LATEX WHICH HAD BEEN HEATED WITH DIETHYLAMINE (2 P.H.R.) IN A SULPHUR-COATED FLASK FOR 24 H AT 70°C, COOLED, COMPOUNDED ACCORDING TO MIX A AND MATURED FOR 2 H AT ROOM TEMPERATURE

PrVn time (h)	Chloroform number	M300 (MPa)		TS (MPa)		EB (%)		V_r at 40°C in toluene UL
		UL	L	UL	L	UL	L	
0	1	0.30	0.25	5.3	3.4	999	943	0.023
10	1	0.34	0.30	5.7	4.2	963	980	0.027
30	2	0.36	0.30	7.7	4.7	996	1 000	0.038
60	2	0.36	0.32	9.4	8.7	989	989	0.054
90	3	0.42	0.44	11.0	8.8	1 005	998	0.061
120	3	0.49	0.44	11.5	9.4	1 003	953	0.065
180	3	0.53	0.50	13.4	9.8	986	999	0.069

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

TABLE 6. PREVULCANISATION AT 70°C OF HA LATEX WHICH HAD BEEN HEATED IN A SULPHUR-COATED FLASK FOR 24 H AT 70°C, COOLED, COMPOUNDED ACCORDING TO MIX A AND MATURED FOR 17 H AT ROOM TEMPERATURE

PrVn time (h)	Chloroform number	M300 (MPa)		TS (MPa)		EB (%)		V_r at 40°C in toluene UL
		UL	L	UL	L	UL	L	
0	1	0.30	0.19	5.6	6.2	1 110	987	0.036
0.5	2	0.41	0.31	12.0	9.4	999	990	0.053
1	3	0.48	0.48	14.7	12.3	1 009	992	0.073
1.5	3	0.52	0.49	16.4	13.1	1 102	993	0.084
2	3	0.59	0.51	16.4	14.6	990	989	0.084
3	4	0.60	0.56	16.9	15.2	1 012	1 109	0.086

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

Maintaining the 17-h maturation period but doubling the incubation period increased the initial rate of crosslinking during subsequent prevulcanisation – indeed, there were indications that some crosslinking was now occurring during the maturation period (*Table 7* and *Figures 2* and *3*). However, there was again a fall in rate after 1 – 1.5 h to a low or zero value. In this case, the much higher limiting values of TS and V_r may be ascribed to dissolution of more sulphur in the latex during incubation. Presumably, saturation of the two latex phases with sulphur is not reached in 48 h.

A feature of the results collected in *Tables 4 – 7* which should not pass un-

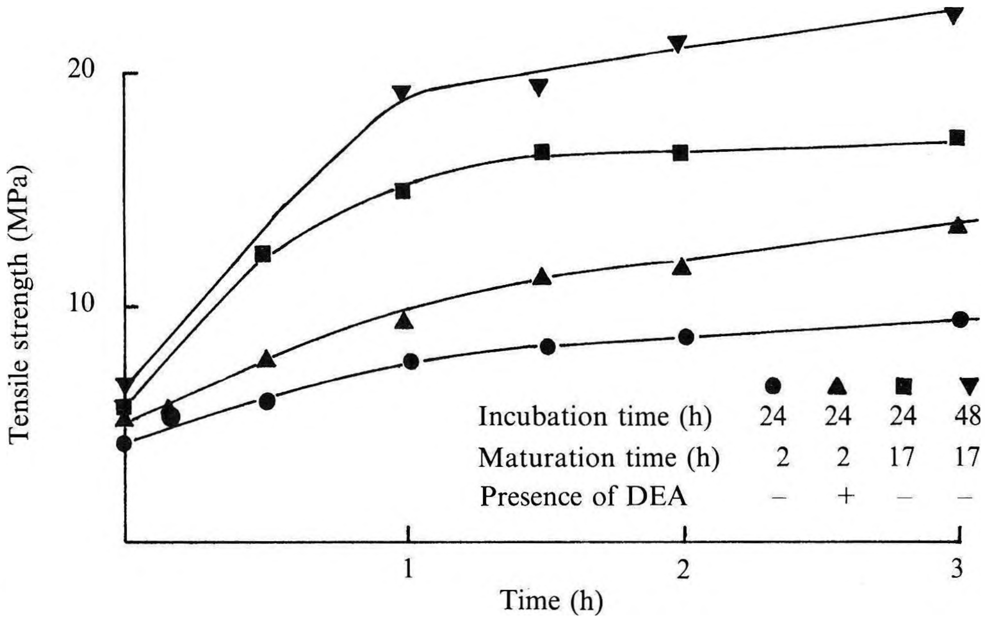

Figure 2. Influence of incubation time and maturation time on the development of tensile strength during prevulcanisation at 70°C of a ZDEC-accelerated system with sulphur present as a solid deposit.

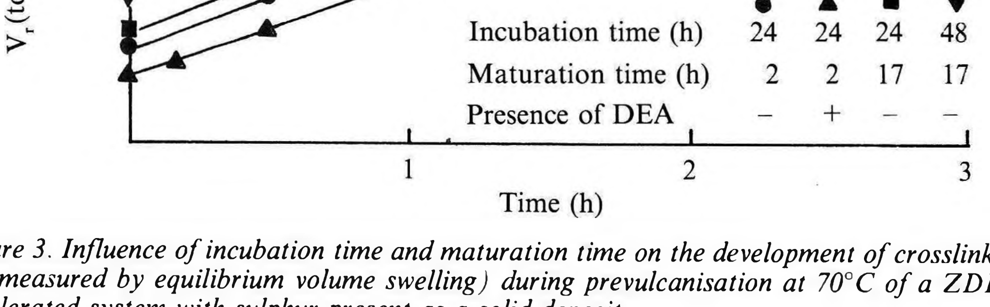

Figure 3. Influence of incubation time and maturation time on the development of crosslinking (as measured by equilibrium volume swelling) during prevulcanisation at 70°C of a ZDEC-accelerated system with sulphur present as a solid deposit.

remarked is the unusual effect of aqueous leaching on the properties of the subsequently dried films. The well-known effects of leaching in increasing strength and, to a lesser extent, stiffness, which have been consistently observed in pre-

TABLE 7. PREVULCANISATION AT 70°C OF HA LATEX WHICH HAD BEEN HEATED IN A SULPHUR-COATED FLASK FOR 48 H AT 70°C, COOLED, COMPOUNDED ACCORDING TO MIX A AND MATURED FOR 17 H AT ROOM TEMPERATURE

PrVn time (h)	Chloroform number	M300 (MPa) UL	M300 (MPa) L	TS (MPa) UL	TS (MPa) L	EB (%) UL	EB (%) L	V_r at 40°C in toluene UL
0	2	0.30	0.28	6.5	6.3	947	843	0.048
1	3	0.42	0.31	19.0	15.1	983	970	0.102
1.5	4	0.42	0.41	19.4	19.7	998	998	0.109
2	4	0.51	0.42	21.1	18.2	997	1 009	0.114
3	4	0.60	0.43	22.2	19.7	999	1 050	0.116

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

vious and concurrent work in these laboratories (see example in Porter and Wong[33]) are absent here. In fact, in many cases, leaching had an adverse effect on tensile strength and modulus. The present lack of understanding of the leaching process prevents any comment being made on the reason for this effect beyond noting that it is presumably related to the unusual procedure used for performing the prevulcanisation.

Incorporation of a Zinc Dithiocarbamate from a Solid Deposit

In spite of the high melting point of ZDEC (175°C), it was possible to spread the liquid material over the wall of a flask without significant decomposition taking place, provided care was taken to avoid overheating. It was found best to pour molten ZDEC from a separate vessel into a cold, rotated flask. Melting ZDEC in the flask to be coated and allowing it to cool did not produce such a robust solid deposit.

When HA latex, compounded as in *Mix C* (*Table 1*), was stirred at 70°C in a ZDEC-coated flask, prevulcanisation took place but only after what appeared to be a long induction period (*Table 8*): the chloroform number was still 1 after 7 h whereas the same mixture containing ZDEC in disper-

TABLE 8. PREVULCANISATION AT 70°C OF HA LATEX, COMPOUNDED ACCORDING TO MIX C, IN A FLASK COATED WITH SOLID ZDEC

PrVn time (h)	Chloroform number	M300 (MPa) UL	M300 (MPa) L	TS (MPa) UL	TS (MPa) L	EB (%) UL	EB (%) L
7.5	1	–	–	–	–	–	–
14.3	4	0.81	1.01	27.1	34.4	911	932
17.7	4	0.83	1.06	25.5	33.3	887	895
24.3	–	0.83	1.16	23.0	31.5	850	895

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

sion attained its maximum tensile strength[33] in 2 h at 70°C. (The TS values in *Table 8* are appreciably higher than those in *Table 9* and in Porter and Wong[33]. This is likely to be because the results in *Table 8* were obtained in a different laboratory and using a cutter and tensile test equipment which were different from those used in obtaining the remaining results in this paper and those in Porter and Wong[33].)

Inclusion of diethylamine in the compounded latex (*Mix D* of *Table 1*) gave the results shown in *Table 9*. After a much shorter induction period (1 – 2 h), prevulcanisation proceeded smoothly for some 4 h, after which M300 of a leached film had reached 0.9 MPa and TS had reached 24 MPa. Thereafter, reaction proceeded more slowly for at least another 12 h, if judged by V_r values (*Figure 4*) (but underwent some reversion if judged by M300).

In view of the results obtained previously, it was anticipated that the presence of sulphur and zinc oxide would not be necessary for the ZDEC to migrate from the solid deposit into the latex. This proved to be the case. Incubation of the latex with ZDEC for 6.5 h in the presence of diethylamine and potassium hydroxide (included to avoid the premature coacervation which had occurred in the experiments with the sulphur deposit) allowed sufficient ZDEC to become incorporated in the latex to obtain rapid and extensive prevulcanisation, after addition of sulphur and zinc oxide (*Table 10*). Indeed, crosslinking appears to be continuing vigorously after 18 h (*Figure 4*). The induction period has disappeared and it is clear that a considerable degree of crosslinking has taken place, if not during the maturation period itself, then during the period before the dried films were tested.

In both experiments with solid deposits of ZDEC, it is noteworthy that tensile strength reaches a maximum after 6 h, at a rather lower modulus than usual[38]. As with the sulphur deposit experiments, aqueous leaching of the vulcanised films had only minor effects on modulus and strength.

DISCUSSION

Sulphur can migrate from a continuous solid deposit where it is present as cyclooctasulphur, S_8, into the rubber particles of HA latex[9]. It has now been demonstrated

TABLE 9. PREVULCANISATION AT 70°C OF HA LATEX, COMPOUNDED ACCORDING TO MIX D, IN A FLASK COATED WITH SOLID ZDEC

PrVn time (h)	Chloroform number	M300 (MPa)		TS (MPa)		EB (%)		V_r at 35°C in toluene	
		UL	L	UL	L	UL	L	UL	L
0	1	0.29	0.37	1.7	4.0	1 050	700	–	–
1	1	0.28	0.37	3.9	3.2	1 050	1 250	–	–
2	1	0.31	0.43	5.5	8.2	1 025	1 100	0.048	0.050
4	2	0.57	0.66	17.2	18.3	1 017	1 050	0.121	0.071
6	3	0.66	0.86	19.8	24.0	1 000	1 050	0.147	0.154
8	3	0.86	0.93	23.2	22.3	1 000	950	0.154	0.151
12	4	0.83	0.89	21.7	20.8	900	950	0.156	0.157
18	4	0.60	0.76	19.0	17.4	850	800	0.166	0.167

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

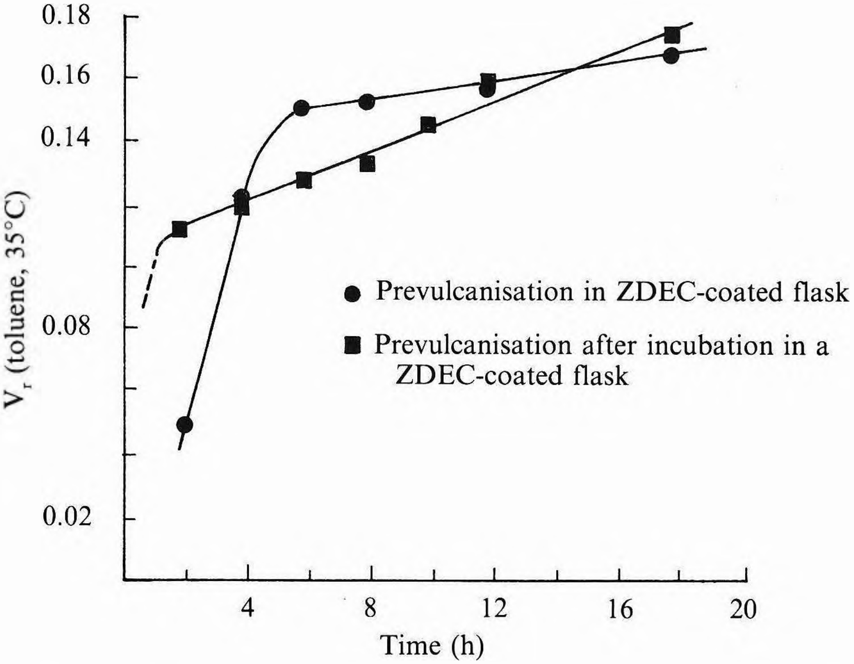

Figure 4. Development of crosslinking (as measured by equilibrium volume swelling) during prevulcanisation at 70°C with ZDEC present as a solid deposit.

TABLE 10. PREVULCANISATION AT 70°C OF HA LATEX WHICH HAD BEEN HEATED WITH DIETHYLAMINE (2 p.h.r.) AND POTASSIUM HYDROXIDE (0.3 p.h.r.) IN A ZDEC-COATED FLASK FOR 6.5 H AT 70°C, COOLED, COMPOUNDED ACCORDING TO MIX E AND MATURED FOR 17 H AT ROOM TEMPERATURE

PrVn time (h)	Chloroform number	M300 (MPa)		TS (MPa)		EB (%)		V_r at 35°C in toluene	
		UL	L	UL	L	UL	L	UL	L
0	1	0.51	0.51	12.9	15.2	1 100	1 150	–	–
2	2	0.63	0.69	21.4	21.7	1 100	1 000	0.119	0.105
4	2	0.75	0.82	22.3	22.9	1 100	1 050	0.121	0.116
6	3	0.74	0.83	21.8	23.4	1 050	1 000	0.128	0.126
8	4	0.75	0.81	20.2	20.3	1 025	950	0.136	0.127
10	4	0.85	0.92	21.8	22.5	950	950	0.146	0.142
12	4	0.85	0.94	21.6	21.5	900	900	0.160	0.158
18	4	0.77	0.94	19.3	20.0	850	875	0.174	0.170

PrVn = Prevulcanisation
EB = Elongation at break
UL = Unleached; L = Leached

that if the latex contains, or is subsequently compounded with, dispersions of a zinc dithiocarbamate accelerator and zinc oxide and heated, the rubber becomes crosslinked. Van Gils[9] finding that the presence of an accelerator or any other compounding ingredient is not necessary for the migration to occur is confirmed; neither is any additional surfactant required. In view of the form of sulphur used in these experiments (a hard deposit), it is difficult to imagine any other transport mechanism for the migration than dissolution in the aqueous serum followed by diffusion into the rubber particles.

There is no evidence that molecular sulphur can dissolve as such in an aqueous system. Sulphur will dissolve in aqueous alkali[39] and is also solubilised by water-soluble and other organic amines such as methylamine[40], dimethylamine[41], morpholine[42], and ethylenediamine[40,42] but in all cases the sulphur is present in solution in the form of open-chain anions: hydrosulphide, HS^-, hydropolysulphide, HS_n^-, and thiosulphate, $S_2O_3^{2-}$, but mainly as polysulphides, S_n^{2-}, polysulphide anions are likely to be the important species in the present context and it may be recalled that the first successful attempts at vulcanising latex were made with alkaline polysulphide solutions[14].

The present experiments shed no light on the nature of the species in which the sulphur enters the rubber particles, nor whether prior reaction with the zinc dithiocarbamate is involved: no evidence has yet been obtained as to whether, in the absence of the dithiocarbamate, the dissolved sulphur remains exclusively in the aqueous phase or whether it becomes distributed between the aqueous and rubber phases in an equilibrium. Van Gils[9] found sulphur present in both phases of his inoculated latices but since he apparently did not re-centrifuge his fractions, his results can be regarded only as indicative.

It does appear from the present incubation experiments (*Figure 3*) that subsequent crosslinking in the rubber phase is limited by the quantity of sulphur taken up by the latex as a whole during incubation. Doubling the time of incubation from 24 h to 48 h increased the equilibrium V_r in toluene from 0.086 to 0.116. While there is strictly no justification for interpreting v_r values obtained on prevulcanised latex films in terms of concentrations of chemical crosslinks (for a variety of reasons), interpretation in this way would indicate an *approximate* doubling of the crosslink density, indicative of a doubling in total sulphur concentration. Presumably, longer incubation times would eventually lead to a limiting sulphur concentration, corresponding to the solubility of sulphur in the whole latex. Clearly, when a zinc dithiocarbamate is present and active during the dissolution of sulphur, the latter becomes combined with the rubber and more dissolves from the solid phase. The degree of crosslinking ultimately reached will then be controlled by other factors.

It has also been shown that a zinc dithiocarbamate can migrate from a continuous solid deposit into dispersed rubber particles and cause crosslinking of the rubber by sulphur. Neither sulphur nor zinc oxide needs to be present for this migration to occur. It is again difficult to envisage any mechanism other than aqueous dissolution for transport of the ZDEC to the rubber particle surface. The enhanced solubility of the dithiocarbamate in latex serum, observed by Loh[10], is presumably sufficient to allow this to occur. Loh's suggestion that the increased solubility is due to complexing of the zinc compound by ammonia or other nitrogenous bases in the serum is supported by the observed effect of diethylamine in considerably shortening the induction period before crosslinking. Diethylamine has been shown to form a 1:1 complex with ZDEC[43] but no information is yet available on the solubility of such complexes in water, nor on their stability towards water.

The principal effect of added diethylamine (a representative secondary amine) in these prevulcanisations appears to be on the dithiocarbamate accelerator rather than on the sulphur. As has been noted, the amine mildly accelerated prevulcanisation of a compounded latex in the presence of a solid sulphur deposit but it had little effect on the uptake of sulphur into HA latex alone (*Table 5*, *cf. Table 4*). In contrast, it had a dramatic effect on the induction period in prevulcanisation with a solid deposit of ZDEC (*Table 9*, *cf. Table 8*).

The present work does not give any information on the extent the dithiocarbamate alone can penetrate into the rubber particles and whether or not it becomes partitioned between the aqueous and polymer phases. If prior reaction with sulphur is necessary to form a rubber-soluble species, ZDEC incorporated into the latex by incubation will remain in the aqueous phase until the second reactant is added. While this situation is thought to be unlikely, it cannot be excluded on present evidence.

In the sulphur incubation experiments, the final extent of crosslinking attained on subsequent compounding and prevulcanisation appeared to reach a limit controlled by the quantity of sulphur which had entered the latex (*Figure 3*). For incubation with ZDEC, there was no evidence for such a limit being reached (*Figure 4*). This is presumably because the ZDEC is largely regenerated during the vulcanisation process so long as zinc oxide is present[2] and remains capable of forming new crosslinks from the sulphur continually entering from the reservoir of the dispersed solid material introduced by the compounding operating.

CONCLUSIONS

The following conclusions are drawn:

- Prevulcanisation of high-ammonia natural rubber latex occurs by prior dissolution of sulphur and zinc dithiocarbamate accelerator in the aqueous serum of the latex.
- Sulphur and accelerator can dissolve independently in the aqueous phase. It is not certain whether they react with one another before penetrating into the rubber particles.
- Latex may be incubated with sulphur and subsequently vulcanised after addition of the accelerator. The degree of prevulcanisation is limited by the amount of dissolved sulphur since no reserve of solid sulphur is present.
- Latex may be incubated with a zinc dithiocarbamate and subsequently vulcanised after addition of a sulphur dispersion. In this case, the degree of prevulcanisation is not limited since the accelerator present is continuously regenerated and remains capable of generating new crosslinks while free sulphur remains.
- Diethylamine, which can catalyse ring-opening (activation) of cyclo-octasulphur and act as a complexing and solubilising agent for zinc dithiocarbamate, appears to exert its main influence on the dithiocarbamate, by drastically shortening the induction period before crosslinking starts.
- The extent of crosslinking attained during prevulcanisation of latex previously incubated with sulphur is sensitive to elapsed time after compounding (maturation time) even though the quantity of available sulphur is fixed.
- Aqueous leaching of rubber films cast from prevulcanised latices in which one ingredient has been incorporated from a solid deposit does not have any significant effect on the stress-strain properties of the films. This behaviour diverges from the enhancement of properties usually observed with films prepared from conventionally prevulcanised latices. The reason for it is not understood.

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Proteins of Natural Rubber Latex Concentrate

H. HASMA*

Proteins of high ammoniated latex concentrate were distributed in two main fractions: the rubber fraction and the serum fraction. The rubber fraction contained primarily the 14 kDa protein, with a minor one at 24 kDa, both of which were extractable by ammonia, sodium dodecyl sulphate and chloroform-methanol. The serum proteins comprised the 14, 24, 29, 36 and 45 kDa proteins and one of molecular weight greater than 100 kDa. These proteins originated from the B- and C-sera of fresh latex and the rubber particles.

Fresh natural latex derived from *Hevea brasiliensis* contains about 0.95% proteins of which 27.2% is in the rubber fraction, 47.5% in the serum fraction and 25.3% in the bottom fraction[1]. In the process of producing centrifuged natural rubber (NR) latex concentrate from which most of the latex articles are fabricated, some of these proteins are lost in the skim latex. Those remaining in the latex concentrate have long been associated with some of the properties of the latex and its products. The mechanical stability of high ammoniated (HA) latex concentrate, for example, is thought to be influenced by the proteins as well as the lipids[2,3]. Proteins have also been shown to influence the stress-strain and modulus of the vulcanised latex concentrate film[4]. Recently proteins in NR latex gloves have been linked to some allergic reactions in human beings[5,6,7].

Although proteins in NR latex have been extensively studied by a number of workers[8-11], their investigations have been mostly confined to the B- and C-sera proteins of fresh latex. Of late, some investigations[12,13,14] have begun on proteins associated with rubber particles (RP), but till now very little attention has been given to the study of proteins in NR latex concentrate. In view of the increasing importance of proteins in this area, the present work was undertaken to investigate the composition of proteins in HA latex concentrate, both adsorbed on the RP and dispersed in the serum fraction.

MATERIALS AND METHODS

Materials

Fresh latex was obtained from mature unstimulated trees. The HA latex concentrate was prepared by centrifuging about 0.5% ammoniated field latex in a De Laval LRH 410-70A centrifugal latex separator. This gave latex concentrate of about 60% dry rubber content which was further ammoniated to 0.7%.

Dialysis tubing of 12 000 molecular weight cut-off and all the chemicals for electrophoresis including the SDS molecular weight markers were purchased from Sigma Chemical Company.

Isolation of Serum Proteins from HA Latex Concentrate

The HA latex concentrate was ultracentrifuged on a Beckman L8-70 ultracentrifuge at 19 000 r.p.m. for about 1 h using rotor 21. The latex fractionated into two layers; the rubber phase and the serum phase with some sludge at the bottom at the centrifuge tube. The milky top layer of the

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serum phase was carefully sucked out and discarded while the clear serum fraction was siphoned using a long needled syringe. The latter fraction was then dialysed against water in the cold for two to three days with frequent changes of fresh water. The dialysed materials were freeze-dried and their weight determined.

Extraction of Proteins from Rubber Particles

Rubber particles (RP) were isolated from fresh latex or HA latex concentrate by ultracentrifugation as above. The isolated RP were rinsed with water two to three times before dispersing them in water, followed by ammoniation to 0.7%, or dispersing them in 2% sodium dodecyl sulphate (SDS). The dispersed RP were stirred for some time and left to stand in the cold overnight. The mixture was then ultracentrifuged and the clear ammonia and SDS extracts were collected as described in the isolation of serum above. The extracts were dialysed against water for two to three days before the dialysed materials were freeze-dried.

In the extraction of proteins from the RP by chloroform-methanol, the isolated RP were first dispersed in a minimum amount of water, filtered through a muslin cloth and the suspension added drop-wise to five volumes of continuously-stirred chloroform-methanol (2:1, v/v) mixture. The extracts, separated from the rubber coagulum, were washed with 0.6% sodium chloride and the resulting mixture separated into three layers on standing in the cold overnight. Any glycoproteins would appear in the top aqueous layer, according to Hamaguchi and Cleve[15]. This fraction was dialysed against water for two to three days, and then freeze-dried. The proteolipids occurred mainly in the interfacial layer, with some in the chloroform layer as reported previously[12]. In this study, the proteolipids were isolated from the interfacial layer by precipitation with acetone and the precipitate dried at room temperature.

Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a linear gradient gel of 8.5% to 17.5% acrylamide with a stacking gel of 3% acrylamide, following the procedure outlined by Boschetti *et al*[16]. Electrophoresis was carried out with 10 μl of sample (40 mg/ml) at a constant current of 30 mA for 5-6 h.

Isoelectric focusing (IEF) was carried out according to the LKB Instruction Note 2217[17] on a 0.5 mm polyacrylamide gel containing ampholine carrier ampholyte pre-blended to pH 3.5 to 9.5, purchased from LKB (Bromma, Sweden). About 10 μl of sample (20 mg/ml) dissolved in 1% glycine was run at a constant power of 25 watts for 45 min with 1*M* sodium hydroxide as cathode electrolyte and 1*M* phosphoric acid as anode electrolyte.

The SDS-PAGE and IEF gels were fixed for 1 h, stained overnight and de-stained several times in solutions prepared according to the LKB Application Note 306[18].

Determination of Sugar and Nitrogen

The glycoprotein fraction was first hydrolysed[19] with 2*N* hydrochloric acid for 2 h and the sugar liberated in the hydrolysate was determined colorimetrically by the Dubois method[20]. The nitrogen content was determined by the Kjeldahl method[21].

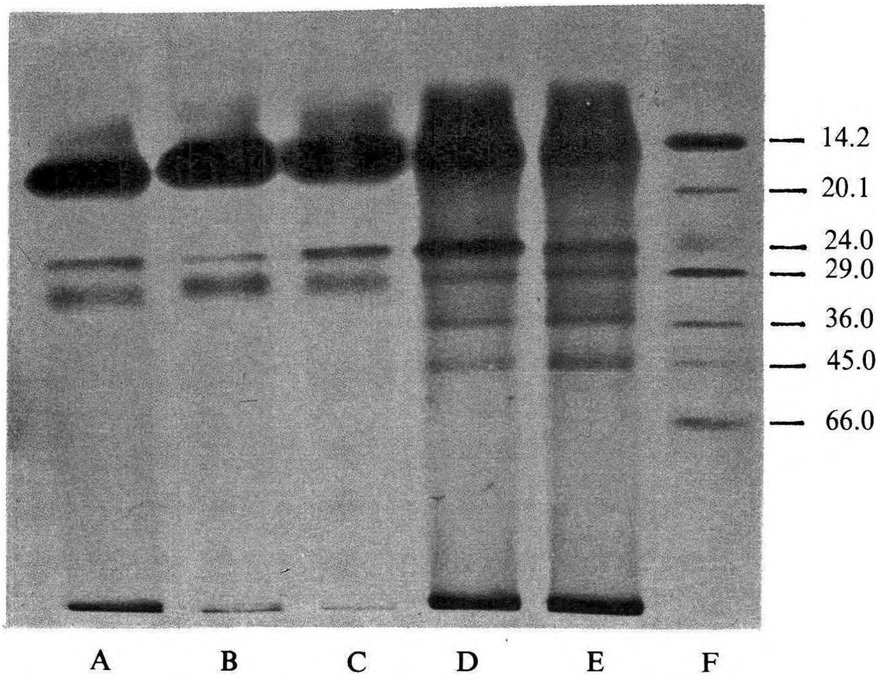
RESULTS AND DISCUSSION

Proteins of HA latex concentrate are distributed between two main fractions; the serum fraction and the rubber fraction.

Proteins in the Serum Fraction

Freeze-drying a dialysed sample of HA latex concentrate serum yielded a brownish powdery material which constituted about 0.51% of the rubber and which contained 12% nitrogen. SDS-PAGE of the sample showed a protein band of a slightly lower mobility than the 14.2 kDa protein marker with some undefined protein bands around the same region, four clear bands at 24, 29, 36 and 45 kDa and another band of very high molecular weight at the boundary between the stacking and separating gels *(Figure 1)*. Some, or all, of these proteins must derive from the B- and C-sera of fresh latex and the rubber particles. The B-serum was incidentally released from the lutoids which burst in ammonia. Previous reports of paper, starch and polyacrylamide gel electrophoresis of B- and C-sera of fresh latex[22,23,24] however showed that there are more protein bands than seen here on SDS-PAGE of the proteins in serum from HA latex. This almost certainly results in part from variations in the methods of analysis. Previous separations were based on differences in both the charge and size of the proteins while the present analysis with SDS-PAGE is based on differences in size or molecular weight of SDS-denatured proteins.

Further analysis on IEF polyacrylamide gel showed that serum from HA latex concentrate indeed contained fewer proteins than the B- and C-sera proteins of fresh latex *(Figure 2)*. The serum proteins of



A. SDS-extracted proteins of RP from 30-day-old RRIM 729
B. Proteolipid fraction of RP from 35-day-old RRIM 600
C. Glycoprotein fraction of RP from 44-day-old PR 255
D. Ammonia-extracted proteins of RP from 48-day-old RRIM 701
E. Serum proteins of 48-day-old RRIM 701 concentrate
F. Molecular weight markers (kDa)

Figure 1. SDS-PAGE of proteins from the rubber and serum fractions of HA latex concentrates.

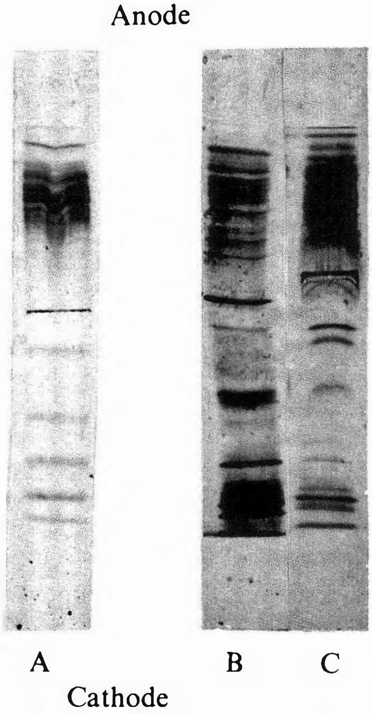


Figure 2. IEF of serum proteins of HA latex concentrate (A) and the proteins from the B- and C-sera of fresh latex (B and C).

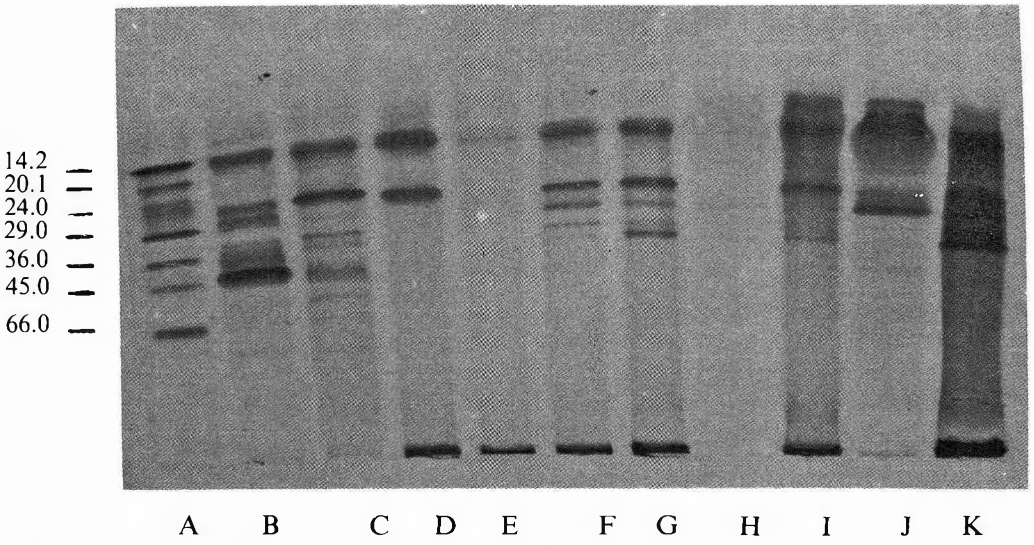
latex concentrate were found to focus mainly in the acidic region with fewer bands in the basic region. This is in accordance with the IEF pattern of the proteins from latex serum prepared by Morales *et al.*[25], by precipitating the rubber in the concentrate with acetic acid. The B- and C-sera proteins of fresh latex however showed a significantly higher number of anionic and cationic protein bands. Thus, in the production of the latex concentrate, a number of proteins may have been lost in the skim latex and sludge, or by denaturation in ammonia as shown by Archer and Sekhar[22].

Proteins in the Rubber Fraction

The proteins associated with the RP could be extracted by ammonia solution, SDS or chloroform-methanol. Extraction by ammonia resembled the process occurring in the storage of latex concentrate and the proteins so extracted are possibly similar to the peripheral proteins of cellular and other membranes, which are known to be easily extracted by changing the pH of the medium[26] e.g. by addition of ammonia[27]. The removal of integral membrane proteins, however, requires detergents and organic solvents[26].

Proteins Extracted by Ammonia

Dispersing the isolated RP of HA latex concentrate in water followed by ammoniation to 0.7%, resulted in an extraction of about 0.1% (gramme per 100 g rubber) of compounds with a nitrogen content of 11%. SDS-PAGE of the extracts showed a protein band at a slightly lower mobility than the 14.2 kDa protein marker, with a few more unresolved bands around the same region, four clear protein bands at 24, 29, 36 and 45 kDa, and another band at the boundary between the stacking and the separating gels *(Figure 1)*. This was in fact similar to the pattern found with serum from HA latex concentrate except for the 14 kDa and 24 kDa proteins, which appeared more concentrated in the ammonia extracts than in the serum. These two proteins probably originated from the RP, while the rest were very likely the proteins of the serum occluded in the rubber during ultracentrifugation. To check this, RP from fresh latex were dispersed both in water and 0.7% ammonia overnight before analysis. The water extracts were found to contain most of the serum proteins, including the 24 kDa protein, but in a lesser amount than the major 45 kDa protein *(Figure 3)*; a 14 kDa protein was also detected. The ammonia extracts, on the other hand, contained mainly the 14 kDa and 24 kDa proteins, besides some serum proteins *(Figure 3)*. In fact, when the RP were re-extracted with ammonia for a second and third time, the 14 kDa and 24 kDa proteins were still present *(Figure 3)*. The 14 kDa protein of the ammonia extracts however ran slightly more slowly than the 14 kDa protein in the water extracts when the mixtures were re-analysed on 15% polyacrylamide gel *(Figure 4)*. The 14 kDa protein in the ammonia extract might have a molecular weight of 14.6 kDa, which is



A. Molecular weight markers (kDa)

B. Proteins from RP of RRIM 730 fresh latex dispersed in water

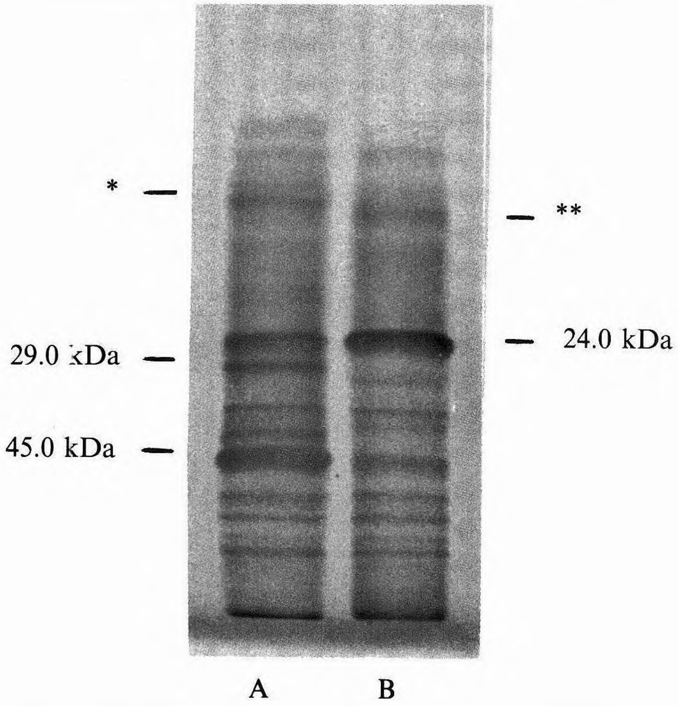
C,D,E. Proteins in the first (C), second (D) and third (E), ammonia extracts of RP from RRIM 730 fresh latex

F,G. Water-soluble (F) and water-insoluble (G) proteins in the ammonia extracts of RP from 4-day-old RRIM 729 latex concentrate

H,I. Water-soluble (H) and water-insoluble (I) proteins in the ammonia extracts of RP from 70-day-old RRIM 729 latex concentrate

J,K. Water-soluble (J) and water-insoluble (K) serum proteins of 24-day-old RRIM 729 latex concentrate

Figure 3. SDS-PAGE of proteins in the ammonia extracts of RP from fresh latex and HA latex concentrate and proteins in the serum fraction of the latex concentrate.



*and** distinguish the two proteins around 14.0 kDa.

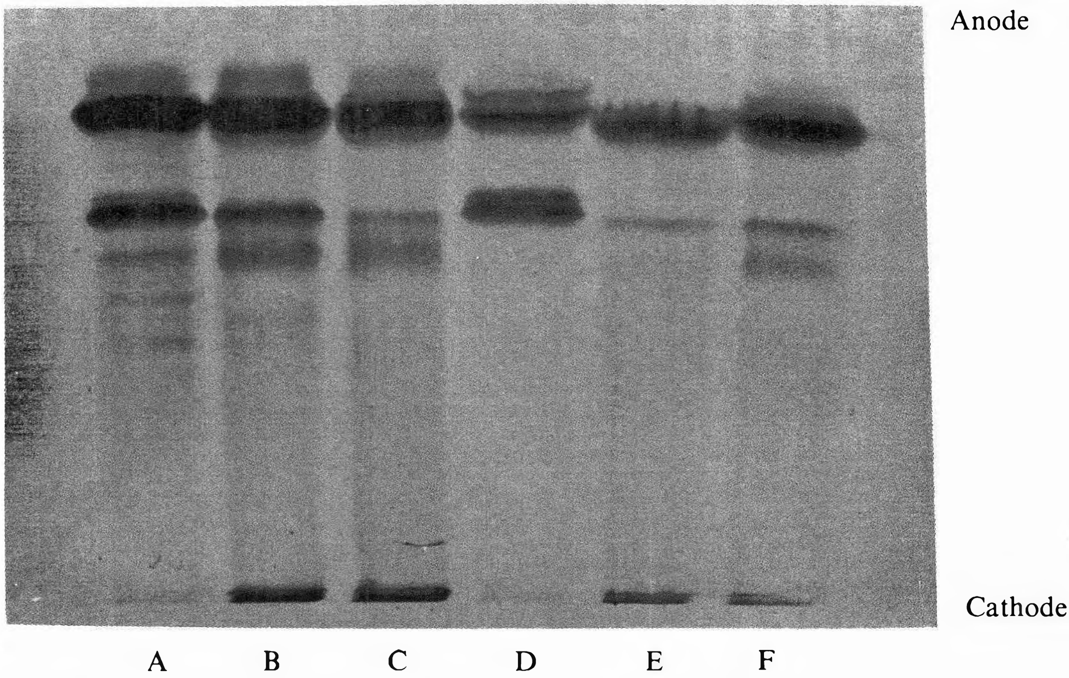
Figure 4. SDS-PAGE of proteins in the water extracts (A) and ammonia extracts (B) of RP from RRIM 730 fresh latex.

the molecular weight of the protein shown by Dennis and Light[3] to occur on the RP, but not in the C-serum of fresh *H. brasiliensis* latex. Thus, ammonia extracted some proteins from the RP besides removing some serum proteins.

It was expected that as the RP were dispersed and stored in ammonia, as in latex concentrate, the 14 kDa and 24 kDa proteins would be progressively removed. The two proteins were however still detectable from the seventy-day-old RP *(Figure 3)*, implying the incomplete or slow extraction of the proteins by ammonia.

Proteins Extracted by SDS and Chloroform-Methanol

A 2% SDS solution was found to extract some membrane components amounting to about 0.3% of the rubber and which contained 10% nitrogen. The extracts were expected to contain some SDS as not all the surfactant could be removed on dialysis. Analysis of the extracts showed one very prominent protein band at 14 kDa, which may again be the 14.6 kDa protein, and two less prominent bands at 24 kDa and 29 kDa *(Figure 1)*. The band at 29 kDa, however, occurred mainly in the extracts of the RP of HA latex concentrate. The SDS extracts of RP from fresh latex did not show this protein band well *(Figure 5)*. A similar extraction of serum-free RP of *H. brasiliensis* latex by Dennis and Light[13] showed a minor 24 kDa protein and a major 14.6 kDa protein [which they termed, the rubber elongation factor (REF)]; no 29 kDa protein was mentioned. So the 29 kDa protein must be derived from the serum fraction of HA latex concentrate, which had earlier been shown to contain this protein *(Figure 1)*. The SDS



A,B,C. SDS-extracted proteins of RP from fresh latex of RRIM 730 (A), and 4-day-old (B) and 60-day-old (C) latex concentrate of RRIM 729

D,E,F. The glycoprotein fraction of RP from fresh latex of PR 225 (D), 10-day-old RRIM 729 (E) and 44-day-old RRIM 701 (F) latex concentrates.

Figure 5. SDS-PAGE of SDS-extracted proteins and the glycoprotein fraction of RP from fresh latex and HA latex concentrate.

extracts of the RP of HA latex concentrates from RRIM 804, RRIM 600, RRIM 729 and RRIM 730, which had been stored for four to sixty days, showed a similar pattern of three protein bands.

A more selective extraction of the RP proteins can be obtained with chloroform-methanol. Washing such extracts of cell membranes with sodium chloride gives a group of proteins at the interface between the chloroform and aqueous layers, which is classified by Folch and Lees[28] as proteolipids. The group of proteins occurring in the aqueous phase from such a procedure are glycoproteins[15].

The SDS-PAGE analysis of the proteolipids from the RP of HA latex concentrate showed a similar pattern to the SDS extracts, with a major band at 14 kDa and two minor ones at 24 kDa and 29 kDa *(Figure 1)*. The pattern however differed from that in the earlier report on proteolipids of RP from fresh unammoniated latex[12]. Proteolipids of such RP gave only two bands, at 14.3 kDa and 24 kDa, as shown in *Figure 6*. As with the SDS extracts, the 29 kDa protein originated from the serum. *Figure 6* demonstrates the insignificant difference in the nature of proteolipids from latex concentrates of different clones and ages.

The amount of proteolipids from the RP of fresh latex of six clones varied from 0.37% to 0.57% of the rubber, while RP matured in ammonia solution gave about

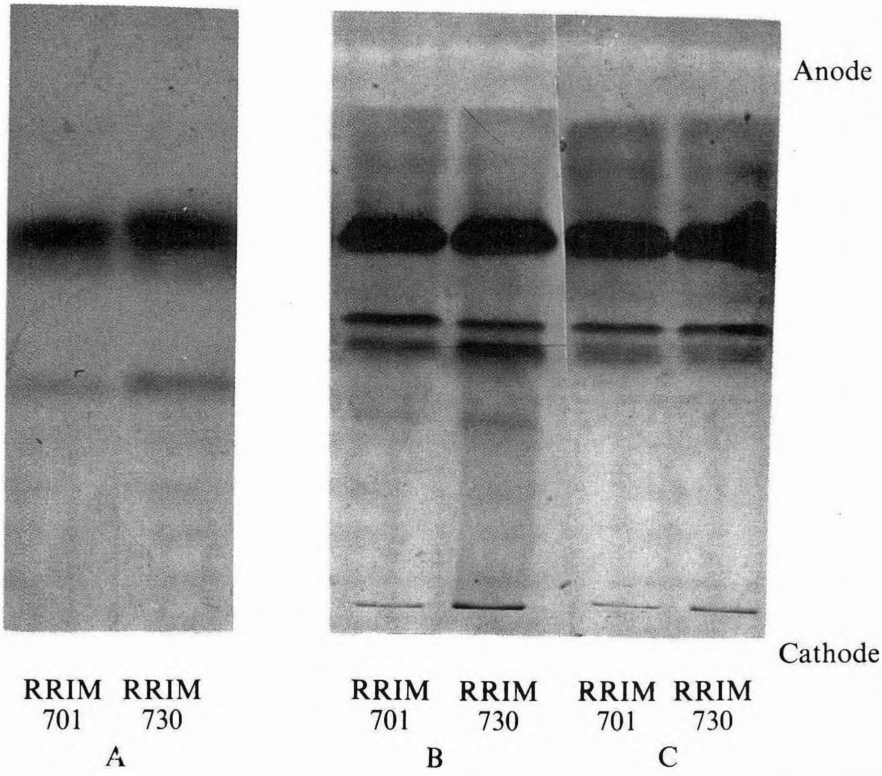


Figure 6. SDS-PAGE of proteolipids of RP from fresh latex (A), and 4-day-old (B) and 35-day-old (C) HA latex concentrates of RRIM 701 and RRIM 730, respectively.

0.40% proteolipids (*Table 1*). Thus the levels of proteolipids from fresh and matured RP were similar. This is not unusual, since integral membrane proteins are not expected to be easily removed by ammonia. The nature of the proteolipids associated with the RP dispersed in 0.7% ammonia of pH 10.0 might however differ from the proteolipids of fresh RP dispersed in C-serum of pH 6.9. This possible difference was not investigated.

As with the SDS extracts and the proteolipid fraction, the glycoprotein frac-

TABLE 1. PROTEOLIPID CONTENT OF RUBBER PARTICLES (RP) FROM FRESH LATEX AND HA LATEX CONCENTRATE OF SIX CLONES

Clone	Amount of proteolipid (% dry weight of rubber)	
	RP of fresh latex	RP of 1-month-old HA latex concentrate
RRIM 730	0.39	0.39
RRIM 701	0.54	0.42
PB 28/59	0.53	0.40
RRIM 600	0.57	-
PR 255	0.37	0.43
RRIM 804	0.54	-

tion from the RP of HA latex concentrate also gave one major protein band at 14 kDa, and two minor ones at 24 kDa and 29 kDa *(Figure 1)*. Again, the 29 kDa protein proved to be serum protein, based on its absence in the glycoprotein fraction of fresh RP *(Figure 5)*. Although SDS-PAGE did not reveal marked differences in the patterns of the proteins in the glycoprotein fraction from the few clones studied (RRIM 730, RRIM 701, RRIM 729, RRIM 600 and PR 255), the amount of material extracted by chloroform-methanol differed significantly with the nature of the latex. Extraction from fresh RP gave about 0.01% to 0.05% (gramme per 100 g of rubber) of materials dissolved in the aqueous fraction *(Table 2)*. The same level was obtained from the RP of HA latex concentrate of less than ten days old. Rubber particles from older latex concentrate, however, gave a much higher level of materials of about 0.23% to 0.35%. It was not ascertained why there were less materials in the glycoprotein fraction from RP of fresh latex than from RP matured in ammonia for more than ten days.

The expected presence of sugar in the glycoprotein fraction was tested for by the Dubois method[20]. The sugar was found to constitute about 1% of the materials in the glycoprotein fraction *(Table 2)*. Preliminary analysis on gas-liquid chromatography showed the glycoprotein fraction to contain only two hexoses, glucose and an unknown sugar, which did not correspond to other sugars tested: *i.e.* arabinose, xylose, galactose, mannose and fucose which are generally known to occur in glycoproteins of biological membranes. Due to very low sugar content associated with the proteins in the glycoprotein fraction of the natural rubber particles, these proteins could not be referred to as glycoproteins, normally found in the membranes of cells and organelles.

Thus the major protein associated with the RP is of molecular weight *ca.* 14 kDa besides the minor 24 kDa protein. The 14 kDa protein is almost certainly the same as the 14.6 kDa REF protein of Dennis and Light[13]. Although these proteins are extractable by different extracting media, namely ammonia, SDS and chloroform-methanol, the current experimental evidence could not implicitly classify them to be different. Furthermore, Dennis and Light[13] showed that there is only one major 14.6 kDa protein and one minor 24 kDa protein in the surface of the rubber particles. Thus, the 14 kDa and the 24 kDa proteins extracted by ammonia and chloroform-methanol may be the same as the ones extracted by SDS.

TABLE 2. AMOUNT OF MATERIAL IN THE GLYCOPROTEIN FRACTION (FOLCH METHOD) FROM RUBBER PARTICLES OF FRESH LATEX AND HA LATEX CONCENTRATES OF SIX CLONES

Clone	Amount of glycoprotein fraction (% dry weight of rubber) Fresh RP[a]	Amount of glycoprotein fraction (% dry weight of rubber) Matured RP[b]	Sugar content of glycoprotein fraction of matured RP[b] (% dry weight of materials in the glycoprotein fraction
RRIM 730	0.02	0.35	-
RRIM 701	0.04	0.25	1.6
PB 28/59	0.01	0.24	0.9
RRIM 600	0.01	0.29	0.9
PR 255	0.05	0.32	1.1
RRIM 804	0.02	0.23	1.8

[a] RP from fresh latex

[b] RP from 30- to 45-day-old HA latex concentrate

CONCLUSION

Proteins of HA latex concentrate were fractionated by SDS-PAGE into six serum proteins (strictly, polypeptides) and two RP membrane proteins. The serum proteins had molecular weights of 14, 24, 29, 36 and 45 kDa and another protein of molecular weight greater than 100 kDa. They form the group of proteins which are most likely to be present in aqueous extracts of latex products. It is to be expected that the water soluble proteins *(Figure 3)* are the most readily leached out.

The proteins associated with the RP are mainly the 14 kDa protein, with a minor 24 kDa protein. These were partially extractable with ammonia solution and thus presumed to be loosely bound to the RP membrane. A greater proportion was tightly bound to the RP and required detergent (SDS), or organic solvent (chloroform-methanol) for its extraction. Such proteins would be expected to remain in the leached latex products. Thus, any influence of proteins on the properties of latex products would be largely attributable to these proteins.

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Stress Relaxation in Natural Rubber Bale Crystallisation During Storage at Low Temperatures

P. BENOIST*, R. CHASSET* AND H. DE LIVONNIERE**

To assess natural rubber crystallisation during storage at low temperatures, measurements were taken using the stress relaxation method. This method makes it possible to assess the overall crystallisation rate over a wide range of temperatures, characterised by a 'melting point' at the Tm *equilibrium point, which is determined by an empirical equation.*

In this study, samples used differed in terms of clonal origin, collection date, processing and drying temperature. A statistical study of these factors showed differences in performance, particularly due to drying temperature. Drying rubber at 125°C rather than 65°C seems to reduce rubber bale crystallisation during storage at low temperatures.

During storage, natural rubber gradually crystallises[1,2], even at temperatures of 5°C to 10°C. This phenomenon can be reversed, and prolonged heating at a sufficiently high temperature enables rubber to recover its original consistency. Experience has shown that the 'background' of natural rubber bales – in both mechanical and heat terms – particularly influences crystallisation. This is the case with residual stress induced by stacking bales on pallets, which constitutes highly propitious sites for crystallisation[3]. Apart from these effects, it was also interesting to look at the effects of the planting material, latex collection conditions and processing treatments at the plantation on the rate of crystallisation of rubber.

Many studies[4,5,6] have shown that the crystallisation process can be broken down into several phases:

- the induction period, corresponding to germ formation
- primary crystallisation, when crystallites develop from the germs
- secondary crystallisation, a slow phenomenon in which no more germs develop and crystallites do not grow any larger, but probably rearrange themselves to form a more perfect crystalline structure.

It is not always possible to study germ formation and crystallite growth separately; an overall study is therefore carried out of the conversion from the amorphous to the crystalline phase. The crystallisation rate is governed by competition between two processes: germ formation, which is faster the lower the temperature, and crystallite development, linked to molecular mobility, which decreases in line with temperature. It therefore depends on temperature[1], and reaches a maximum, as shown in *Figure 1*.

Crystallisation is accompanied by variations in some physical properties of the material, each of which can be used to measure crystallinity. This study used the stress relaxation method. In effect, if the polymer is kept under a constant strain, crystallisation leads to an additional gradual

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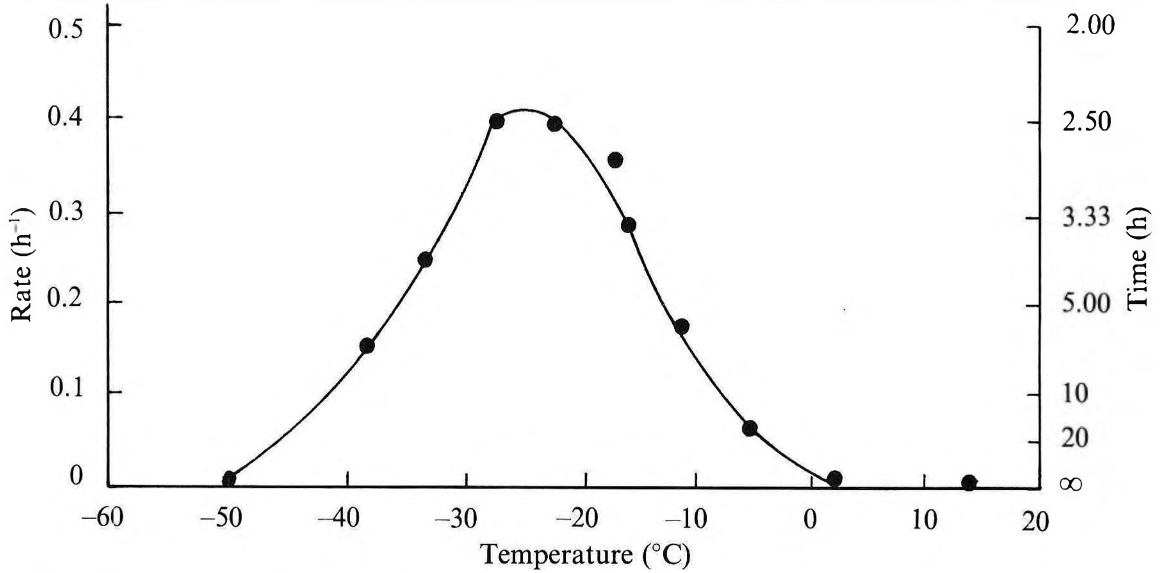

Figure 1. Natural rubber crystallisation rate $(1/t^{1/2})$ versus *temperature (according to L.A. Wood and N. Bekkedahl*[1].

stress relaxation, which also makes it possible to study the phenomenon over time.

MATERIALS AND METHODS

Definition of the Rubbers Studied

The rubbers were prepared at the IRCA Experimental Station in the Ivory Coast. Two clones were chosen: GT 1 and PR 107. All the latex was collected from the same groups of trees *Block* A_3 for GT 1 and *Block* D_7 for PR 107 – at two different periods: July and November. Three latex coagulation methods were used:

- *Method I* : Initial d.r.c., acidification to pH 5.2, 16 h maturing. *Method I* is the most commonly used method in the Ivory Coast.
- *Method II* : 15% d.r.c., coagulation by 10 g of acetic acid/kg of dry rubber (pH 4.5 after acidification), 16 h maturing. *Method II* is used to coagulate smoked sheets (RSS) or air-dried sheets.
- *Method III* : Initial d.r.c., natural coagulation for four weeks in polythene bags under *Hevea* cover. *Method III* is a simulation of the coagulum collection method used by smallholders.

After coagulation, the rubber was processed as follows: dry crumbing in *Methods I* and *II*, creping (fed through ten times under water), followed by wet crumbing in *Method III*. The crumb was then split into two batches; one of which was dried at 65°C and the other at 125°C.

Table 1 gives the conditions for preparation of the main rubber samples and some characteristics of the raw gum produced: Mooney viscosity *(MV)*, Wallace plasticity (P_0), plasticity retention index *(PRI)*, nitrogen and volatile matter content size as per standard *ISO 2000*. The average molecular weight $(\bar{M}v)$, determined by viscosimetry*, is also given.

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*Measurements were made at 25°C in toluene: based on the intrinsic viscosity value [ ], $\bar{M}v$ was calculated according to the equation [ ] = $K\bar{M}v^a$, where $K = 5.02 \times 10^{-4}$ and $a = 0.667$

TABLE 1. CONDITIONS OF PREPARATION AND SOME CHARACTERISTICS OF THE RUBBERS STUDIED

Rubber sample	Clone	Latex collection date	Coagulation method	Drying temp. (°C)	M.V.	P_o	PRI (%)	N2 cont. (%)	Volatile matter (%)	$\overline{M}v \times 10^{-6}$
22-1	GT 1	July	II	65	83	51	69	0.45	0.31	1.10
23-1				125	85	49	79	0.45	0.20	0.99
24-4		November	III	65	94	56	43	0.26	0.54	0.97
24-4B				125	83	31	31	0.23	0.25	0.56
30-2	PR 107	November	I	65	85	51	76	0.51	1.20	1.11
31-2				125	94	51	74	0.51	1.01	0.92
32-1		July	II	65	82	53	80	0.39	0.22	1.15
33-1				125	86	51	83	0.43	0.19	1.00
32-2		November	II	65	82	50	79	0.44	0.59	1.08
33-2				125	89	53	76	0.44	0.59	0.72
34-2		November	III	65	95	58	47	0.25	0.27	1.23
35-2				125	77	36	17	0.25	0.18	0.735

Stress Relaxation

Description of the relaxometer. This apparatus has already been used several times in previous studies[7,8]. It comprises an atmospheric chamber ensuring a constant temperature – accurate to 0.2°C – within a range of −30°C to +10°C. The test-pieces are placed under strain rapidly: less than $^1/_{10}$ s for extensions of 50% or less. Tensile load is measured using a stress gauge sensor. The apparatus is microcomputer-controlled, ensuring direct data input and processing.

The test-piece is a 50 mm long parallelepiped with a cross-section of 4 × 2 mm², with two rectangular end-pieces, which are fixed into stirrup clamps. This prevents the pieces slipping during the trial, as proved by a cathetometer study. The test-pieces are punched from a block of homogenised rubber *(ISO 1796)*, moulded at 100°C in a heat cycle which cancels out the rubber's 'mechanical background' and destroys most of the residual stress and crystallite germs.

Relaxation kinetics. If there is no crystallisation, the relaxation kinetics of non-vulcanised rubbers follow a relationship of the following type, over several decade periods:

$f=f_{st-n}$ (where n is constant).

The curves obtained have a linear part on a double logarithmic scale, which slopes more sharply the higher the temperature: molecular chain slipping in relation to one another – the main cause of stress relaxation – is favoured by increased temperature. When the temperature decreases, the curve points downwards, since the chain segments are gradually incorporated into the crystallites and are no longer part of the elastic reaction. At the end of crystallisation, tensile load tends towards a nil value. *Figure 2* gives an example of these curves, with variations over time in the $F = f/f_1$ ratio, where f is the strength at time t and f_1 the strength of the test-piece after 1 min strain. Time t_1 was chosen as the start of crystallisation; it corresponds to the point when deviation compared with the linear relationship is first observed. It is then easy to determine the half crystallisation time : $t^1/_2 = t_2 - t_1$ (if F_1 is the value of f/f_1 at t_1, t_2 is the time for which $F_2 = {}^1/_2F_1$). It gives an overall characterisation of the rate of crystalli-

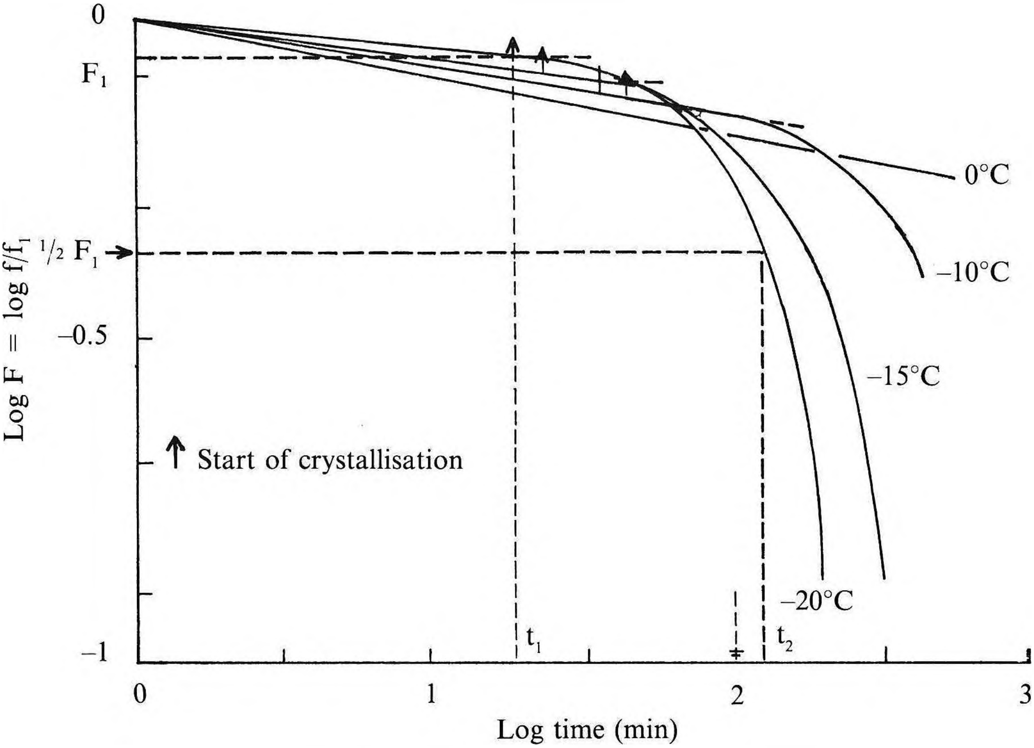

Figure 2. Stress relaxation curves for a non-vulcanised natural rubber.

sation under good conditions of reproducibility.

Exploitation of results (Bruzzone and Sorta's equation). Particular attention was paid to a quantitative description of the variations in the overall rate of crystallisation, estimated by half-time $t^1/_2$, depending on the crystallisation temperature Tc. Based on previous theoretical work[9] on non-stressed polymers, Guiliani, Bruzzone and Sorta[10,11] proposed a semi-emprical equation:

$$\log \frac{t^1/_2}{a\tau} = a + b \frac{Tm}{Tc\ (Tm - Tc)}$$

where a and b are constant. Temperature Tm is known as the 'melting point' at the point of equilibrium; $a\tau$ designates the reduction factor proposed by William *et al.*[12]:

$$\log a\tau = - \frac{8.86\ (T - Ts)}{101.6 + T - Ts}$$

where Ts is conventionally a temperature characteristic of each polymer and equal to Tg + 50K. By applying this equation to the rate of crystallisation of eight different polymers, including natural rubber, the authors found that all the experimental data could be correctly represented if the same values were taken for a and b, irrespective of the polymer:

$$a = -4.61,\ b = 338.$$

Under these conditions, Tm becomes an adjustable parameter making it possible to simply characterise the rate of crystallisation of the sample in question. It is worth noting that the Tm values determined in this way are higher than those given in literature, as pointed out by Kim and Mandelkern[9]. It is possible that the differences between the two sets of determinations could be explained by the different ways in which the equilibrium was prepared.

Using Bruzzone and Sorta's equation, a set of $t^{1}/_{2}$ values were measured at x crystallisation temperatures $(Tc)_i$, on a polymer sample. To assess the melting point Tm of a sample, it is possible to calculate the $(Tm)_i$ corresponding to each temperature $(Tc)_i$, simply by applying Bruzzone and Sorta's equation and then taking the average of the $(Tm)_i$ values determined. The distribution of results can be explained by the standard deviation σ in the distribution of $(Tm)_i$ values.

EXPERIMENTAL RESULTS

Effect of Extension

Variations in $t^{1}/_{2}$ depending on extension of between 10% and a maximum of 100% were determined on *Sample 22-1*. As extension favours macromolecular chain orientation, $t^{1}/_{2}$ values are lower the greater the test-piece extension. This means that the rate of crystallisation $1/t^{1}/_{2}$ is faster the greater the extension, as shown in *Figure 3* at three temperatures: –10°C, –15°C and –20°C. In addition, there is a close correlation between log $1/t^{1}/_{2}$ and extension, which tallies with Gent's observations[13].

Hence, for the rest of the study, a relatively moderate uniform strain of 30% was taken so as not to alter the classification

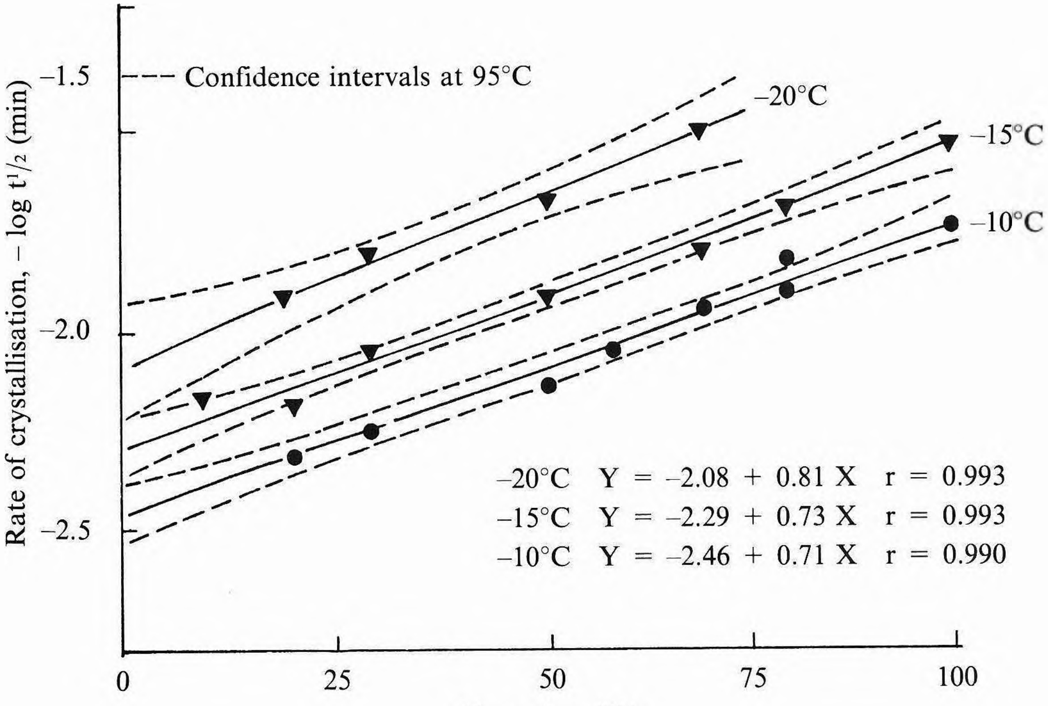

Figure 3. Effect of extension on rate of crystallisation of rubber sample 22-1.

of the various rubbers examined, but enough to accurately measure strength variations over time.

Rate of Crystallisation of the Samples Studied

For each of the above natural rubber samples, the half crystallisation time $t^{1}/_{2}$ were measured by stress relaxation, depending on the temperature within a range of –27°C to –5°C, the upper limit depending on the rate of crystallisation.

Based on these experimental data and using Bruzzone and Sorta's equation, as in the above procedure, it was possible to give each sample a melting temperature *Tm*, for which values are given in *Table 2*, in addition to the standard deviation σ of the $(Tm)_i$ values, the coefficient of distribution *e*%, the $t^{1}/_{2}$ value (min), calculated at –5°C and the rate $V = 1/t^{1}/_{2}$ (day^{-1}), again at –5°C. Examination of *Table 2* shows a very wide distribution of the standard deviation σ, which ranges from 0.23 *(Sample 24-4)* to 1.64 *(Sample 32-2)*, a marked Gaussian distribution which is difficult to explain except by the inevitable sources of error when taking particularly difficult measurements. Nevertheless, the curves in *Figure 4* show that a melting temperature *Tm*, based on Bruzzone and Sorta's equation, makes it possible to satisfactorily represent experimental results, except for *Sample 32-2*, where there is a certain distortion, and – to a lesser degree – for *Sample 30-2*. In addition, variations in temperature *Tm* are relatively large: 5° (from 311.8K for *Sample 25-4B* to 316.8K for *Sample 32-2*), corresponding to rates of crystallisation at –5°C which can vary within a ratio of 1:6.2. Based on *Tm* values and distribution, it is worth looking at the effect – on a statistical level – of certain natural rubber production factors on the rate of crystallisation.

'Clone' effect. Only two clones were considered in this study: GT 1 and PR 107, with only one *'sheet'* type coagulation method *(Method II)*: *Samples 22-1* and *32-1* (dried at 65°C) and *Samples 23-1* and *33-1* (dried at 125°C). *Table 3* gives a statistical analysis of the results based on a comparison of mean *Tm* values, according to Student's method, which uses the *'t test'*.

TABLE 2. RESULTS OBTAINED BY APPLYING BRUZZONE AND SORTA'S EQUATION TO THE RUBBERS STUDIED

Rubber sample	$\bar{T}m$ (K)	σ	e%	$t^{1}/_{2}$ at –5°C (min)	$v = 1/t^{1}/_{2}$ at –5°C (days^{-1})
22-1	315.8	1.22	0.39	245	5.88
23-1	312.1	1.09	0.35	960	1.50
24-4	313.0	0.23	0.07	675	2.13
25-4B	311.8	0.59	0.16	1085	1.33
30-2	314.6	1.26	0.40	375	3.84
31-2	313.6	0.52	0.16	540	2.67
32-1	316.0	1.15	0.36	230	6.26
32-2	316.8	1.64	0.52	175	8.22
33-1	312.5	0.47	0.15	820	1.76
33-2	315.4	0.67	0.21	280	5.14
34-2	316.7	0.83	0.26	180	8.00
35-2	312.2	0.42	0.13	925	1.56

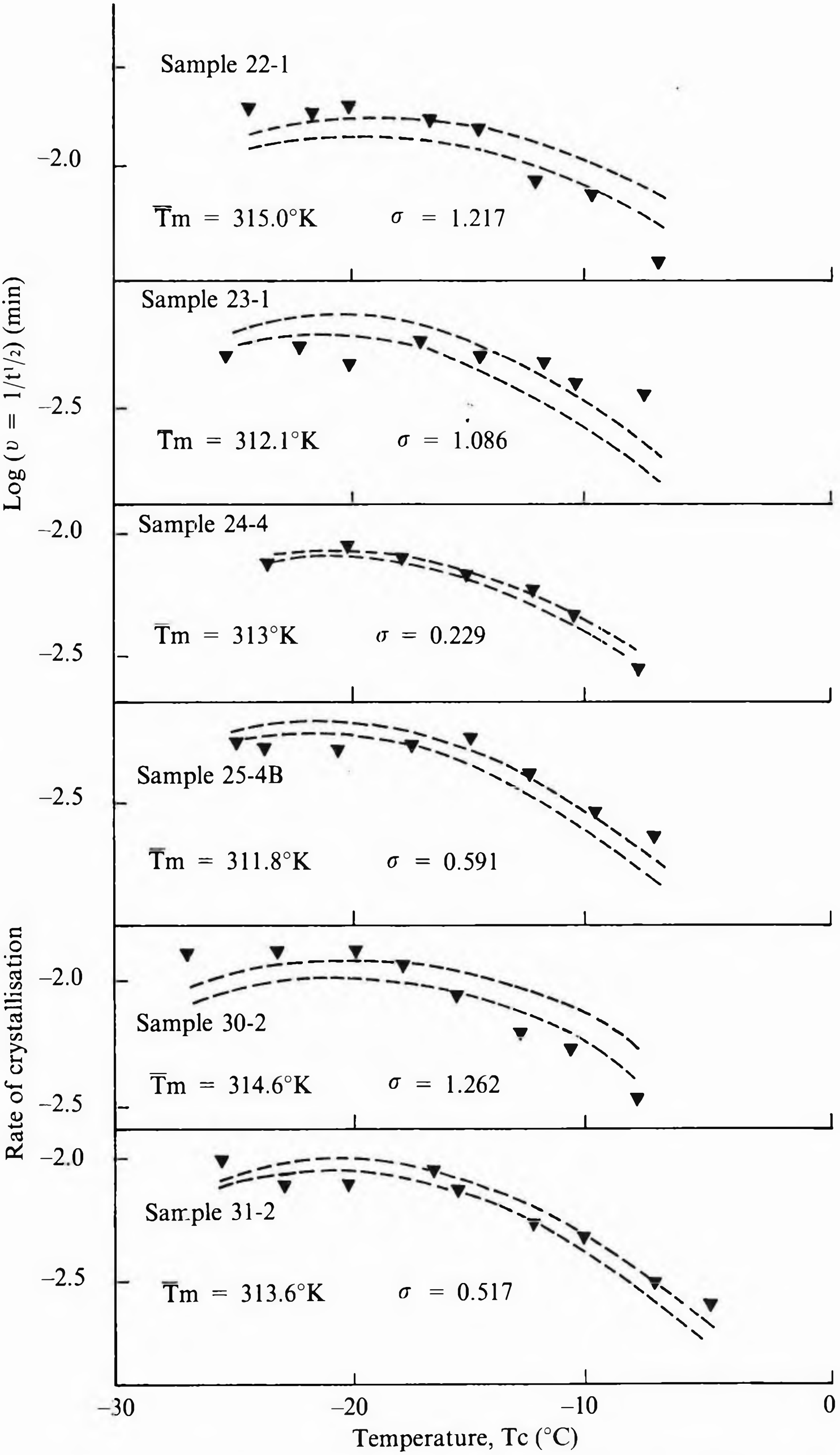

Figure 4. Bruzzone and Sorta's equation applied to experimental results: rubber samples 22-1, 23-1, 24-4, 25-4B, 30-2, and 31-2,

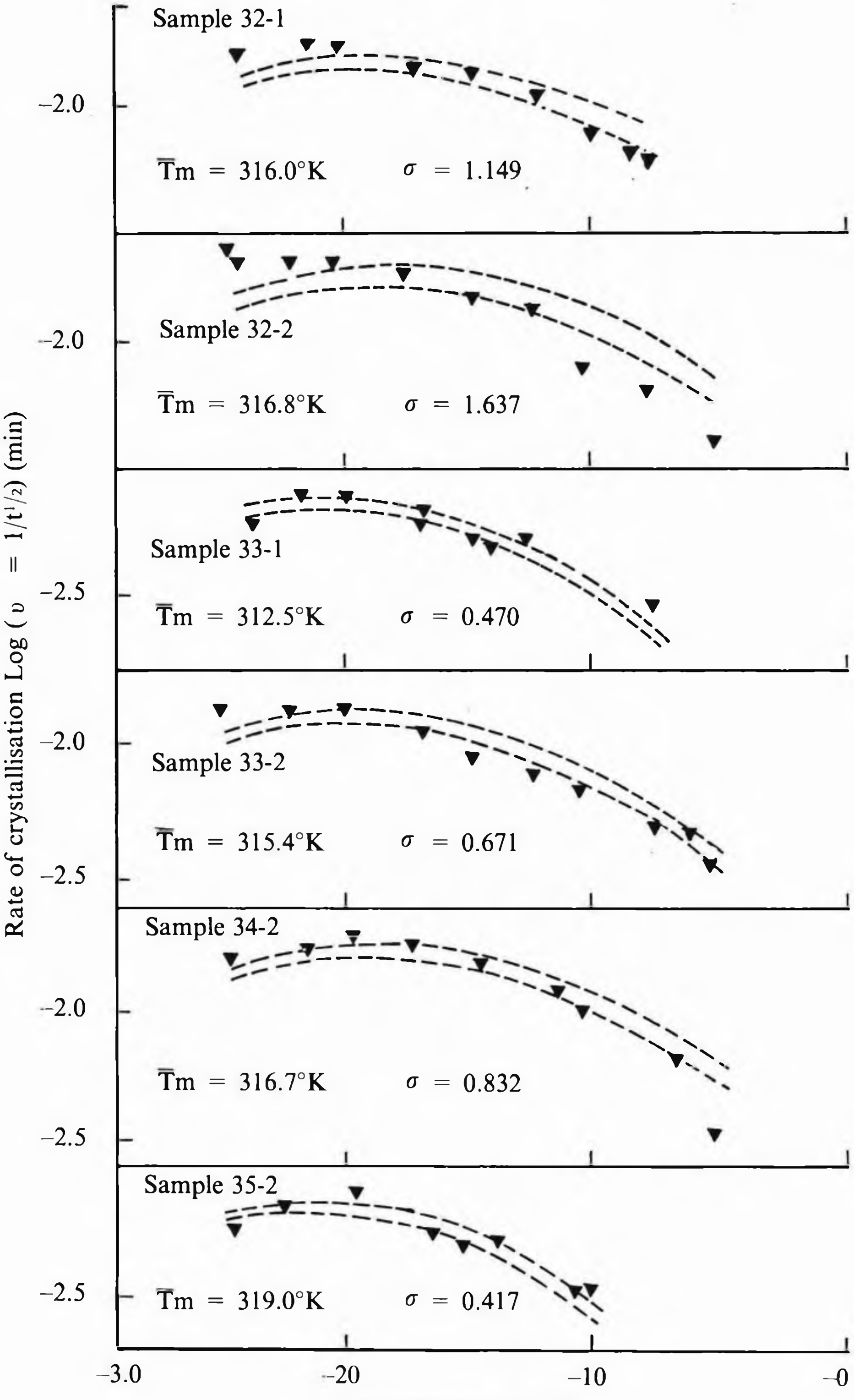

Figure 4 (contd.) Bruzzone and Sorta's equation applied to experimental results: rubber samples 32-1, 32-2, 33-1, 33-2, 34-2 and 35-2.

TABLE 3. STATISTICAL ANALYSIS – 'CLONE' EFFECT

Rubber sample	Clone	Drying temp. (°C)	$\bar{T}m$ (K)	σ	n	t	∅	Significance	v (d^{-1}) at –5°C
22-1	GT 1	65°C	315.8	1.22	8	0.35	15	Not significant	5.88
32-1	PR 107		316.0	1.15	9				6.26
23-1	GT 1	125°C	312.1	1.09	8	1.01	15	Not significant	1.50
33-1	PR 107		312.5	0.47	9				1.76

Under the experimental conditions in question, there was no significant difference between the rubbers produced from GT 1 and PR 107 latex.

'Collection date' effect. This study also only involved a limited number of samples, all produced from clone PR 107, *'sheet'* type coagulation *(Method II)*: *Samples 32-1* and *32-2* (dried at 65°C) and *Samples 33-1* and *33-2* (dried at 125°C). The statistical analysis is given in *Table 4*. There is no difference between the two samples dried at 65°C. However, for the samples dried at 125°C, the rubber prepared in July crystallised about three times less quickly than the rubber collected in November of the same year.

'Coagulation method' effect. The six samples considered came from the same clone – PR 107 – and corresponded to the three methods of coagulation studied:

- *Method I* : *Samples 30-2* and *31-2*
- *Method II* : *Samples 32-2* and *33-2*
- *Method III* : *Samples 34-2* and *35-2*

Three rubbers (*Samples 30-2, 32-2* and *34-2*) were dried at 65°C and the others *(Samples 31-2, 33-2* and *35-2*) at 125°C. *Table 5* gives a statistical analysis of the results.

There are highly significant differences, except for the *Samples 32-2/34-2* pair, dried at 65°C after coagulation as in *Method II* and *Method III* respectively. In other words, based on the rate of crystallisation at – 5°C, it is possible to sum up the 'coagulation method' effect as follows:

Drying at 65°C	Drying at 125°C
$v_{II}\ v_{III} > v_I$	$v_{II} > v_I > v_{III}$

Taking the lowest rate – *Sample 35-2* – as a reference, the following quantitative classification is obtained (in increasing order of rate):

v_{III} = 1)
v_I = 1.7) dried at 125°C
v_{II} = 3.3)
v_I = 2.5) dried at 65°C
$v_{II}\ v_{III}$ = 5.2)

TABLE 4. STATISTICAL ANALYSIS – 'LATEX COLLECTION DATE' EFFECT

Rubber sample	Latex collection date	Drying temp. (°C)	$\bar{T}m$ (K)	σ	n	t	∅	Significance	v (d^{-1}) at –5°C
32-1	July	65	316.0	1.15	9	1.24	18	Not significant	6.26
32-2	November		316.8	1.64	11				8.22
33-1	July	125	312.5	0.47	9	10.8	17	Highly significant	1.76
33-2	November		315.4	0.67	10				5.14

TABLE 5. STATISTICAL ANALYSIS – 'COAGULATION METHOD' EFFECT

Rubber sample	Coagulation method	Drying temp. (°C)	$\overline{T}m$ (K)	σ	n	t	∅	Significance	v (d^{-1}) at –5°C
30-2	I	65	314.6	1.26	9	3.30	18	Highly significant	3.84
32-2	II		316.8	1.64	11				8.22
30-2	I		314.6	1.26	9	4.17	16	Highly significant	3.84
34-2	III		316.7	0.83	9				8.00
32-2	II		316.8	1.64	11	0.17	18	Not significant	8.22
34-2	III		316.7	0.83	9				8.00
31-2	I	125	313.6	0.52	9	6.50	17	Highly significant	2.67
33-2	II		315.4	0.67	10				5.14
31-2	I		316.6	0.52	9	6.10	15	Highly significant	2.67
35-2	III		312.2	0.42	8				1.56
33-2	II		315.4	0.67	10	11.8	16	Highly significant	5.14
35-2	III		312.2	0.42	8				1.56

'Drying temperature' effect. Six sets of two samples, one dried at 65°C and the other at 125°C, were examined. Two sets were from clone GT 1, coagulated as in *Method II* or *Method III;* the other four sets were from clone PR 107 coagulated as in *Methods I, II* and *III.* For *Method II,* latex collected at two different times was used *(Table 6). Table 6* shows that irrespective of the clone, and allowing for the coagulation method

TABLE 6. STATISTICAL ANALYSIS – 'DRYING TEMPERATURE' EFFECT

Rubber sample	Drying temp. (°C)	Coagulation method	$\overline{T}m$ (K)	σ	n	t	∅	Significance	v (d^{-1}) at –5°C
22-1	65	II	315.8	1.22	8	6.41	14	Highly significant	5.88
23-1	125		312.1	1.09	8				1.50
24-4	65	III	313.0	0.23	7	5.03	13	Highly significant	2.13
25-4B	125		311.8	0.59	8				1.33
30-2	65	I	314.6	1.26	9	2.20	16	Significant	3.84
31-2	125		313.6	0.52	9				2.67
32-1	65	II	316.0	1.15	9	8.46	16	Highly significant	6.26
33-1	125		312.5	0.47	9				1.76
32-2	65	II	316.8	1.64	11	2.51	19	Significant	8.22
33-2	125		315.4	0.67	10				5.14
34-2	65	III	316.7	0.83	9	13.8	15	Highly significant	8.00
35-2	125		312.2	0.42	8				1.56

and the collection date, rubbers dried at 65°C crystallise more rapidly than those dried at 125°C. The differences observed are generally highly significant, except for two cases, where they are only significant:

- *Samples 30-2/31-2* PR 107 latex collected in November, coagulated as in *Method I*
- *Samples 32-2/33-2* PR 107 latex collected in November, coagulated as in *Method II.*

In quantitative terms, the mean rates of crystallisation at –5°C were 5.72 and 2.33 for the samples dried at 65°C and 125°C respectively. On the whole, rubbers dried at 65°C crystallise about 2.5 times faster at –5°C than those dried at 125°C.

DISCUSSION

The study of the 'drying temperature' effect involved twelve rubbers; it has been seen that: $v_{65^\circ C} > v_{125^\circ C}$ with a ratio of 1:2.5. It is worth comparing this observation with the correlation between the melting point at equilibrium *Tm*, which gives an overall characterisation of rate of crystallisation, and average molecular weight $\overline{Mv}$ *(Figure 5)*: the greater the $\overline{Mv}$ value, the higher the *Tm* value, corresponding to a rubber which crystallises more slowly. In fact, all else being equal, drying at 125°C

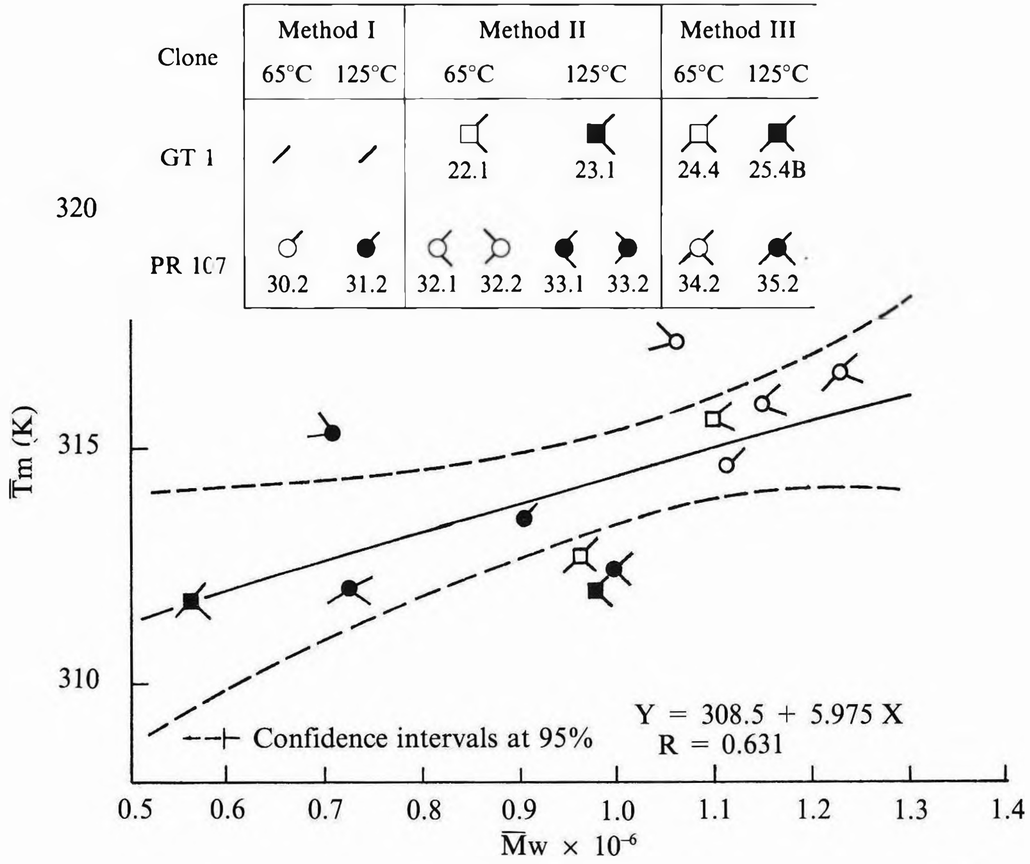

Figure 5. Correlation between melting point $\overline{Tm}$ and average molecular weight $\overline{Mv}$.

reduces molecular weight to some extent, as shown in *Table 1* and *Figure 5*. This reduction in $\overline{M}v$ leads to a larger number of chain ends, which act as a dilutant as crystallisation develops[5], hence reducing the rate of crystallisation. In other words, drying rubbers at 125°C is worthwhile, since it tends to slow crystallisation during storage at low temperatures, without altering the mechanical properties of vulcanisates. As far as the other effects are concerned, differences are harder to find.

The study of the 'coagulation method' effect only considered clone PR 107. The statistical analysis showed that irrespective of drying temperature $v_{II} > v_{I}$ (can be as much as double), rubber coagulated as in *Method III* and dried at 65°C crystallises rapidly (as with *Method II*), whereas rubber dried at 125°C crystallises more slowly.

Methods I and *II* differ in terms of latex dilution and the more acidic medium used in *Method II* (where the pH is 4.5 after acidification as against 5.2 for *Method I*). The lower the pH, the higher the non-rubber element content, and non-rubber elements, particularly fatty acids, are all possible germination sites and crystallisation triggers, which could explain the different rates of crystallisation observed between rubber prepared by *Methods I* and *II*. To confirm this hypothesis, the method should be developed so as to systematically titrate fatty acids. However, it is difficult to explain the classification of samples coagulated naturally *(Method III)*.

As far as the 'latex collection date' effect is concerned, too few trials were carried out. Whereas there is no difference between the two rubbers dried at 65°C, the samples dried at 125°C perform differently: the rubber produced from latex collected in July (v = 1.76 days^{-1}) crystallises around three times less quickly than rubber prepared in November (v = 5.14 days^{-1}). According to monthly rainfall records, 1636 mm of rain fell in the three months up to the July collection, whereas only 159 mm fell during the three months up to November. Although this had no notable effect on d.r.c., this significant difference in rainfall necessarily affected the water balance in the tree and consequently latex composition. However, this phenomenon has not yet been explained.

As regards the 'clone' effect, there is no difference between GT 1 and PR 107 with respect to the treatments studied. It is worth noting that Bristow and Sears[3] mentioned that by looking at a larger number of clones, they had detected a degree of clone effect, but as all commercial rubbers are blends of various clones, this is of no practical relevance.

CONCLUSION

The stress relaxation method seems to be appropriate for measuring the rate of crystallisation of natural rubber during storage at low temperatures, and can easily be used to record trial results. The study of samples prepared on plantations under clearly defined conditions made it possible to put foward hypotheses on seasonal and coagulation method effects. It was also shown that drying at high temperatures, for example 125°C, tends to slow the rate of crystallisation.

From a practical point of view, the effect of these various factors combines with that of residual mechanical stress within the rubber bales, due to actual storage conditions.

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A 100-year Case Study of the Life Prediction of Natural Rubber for an Engineering Application

KAMARUDIN AB. MALEK* AND A. STEVENSON**

A thick rubber block was obtained after ninety-six years' ageing in air. The rubber was analysed for its formulation. Thick rubber blocks were prepared using this formulation and they were subjected to different periods of ageing and different temperatures. Puncture tests were performed on these aged samples and a characteristic time t_a was obtained for different ageing temperatures. Arrhenius plots of characteristic times of samples aged up to ten years and from that of the ninety-six-year-old sample were found to be linear.

Rubber engineering components are frequently bulky and include thick layers of rubber. These components are sometimes expected to last for a very long time – e.g. over 100 years for civil engineering applications. Therefore, it is necessary to have some method of assessing the probable life-time of these rubber components at the design stage. This paper describes the use of a puncture test[1,2,3] to investigate changes in thick rubber blocks subjected to elevated temperatures and long-term ageing. The test consists of indentation and rupture of the surface of a rubber block by a cylindrical indentor as shown schematically in *Figure 1*.

The strength of rubber can be seriously affected by oxidation and the effect becomes worse when the rubber is subjected to high temperatures. It is known that a small amount of oxygen (*ca.* 1%) absorbed by a natural or synthetic rubber can have a deleterious effect on its physical properties[4]. Much research has been concerned with the mechanisms of the basic oxidation processes, namely the interaction between elemental oxygen and rubbers. The understanding of these chemical processes has been derived largely from the work on model systems and generally, on thin sheets of vulcanisate[5].

From an engineering point of view, the chemistry of oxidation has not yet answered the question of how long a bulky rubber component will last in service conditions, especially at elevated temperatures. Conventional ageing tests use thin test-pieces to characterise mechanical properties such as stiffness and strength. The properties are usually measured after oven-ageing for a specific time at a specific temperature. The ageing behaviour of thin rubber test-pieces may not represent that of thick blocks or indeed even that of the surface of thick blocks. This is because of the inhomogenous nature of high temperature ageing of thick rubber blocks affecting mainly the surface[3,6]. The difference in strength between the surface and the bulk rubber can be easily evaluated by using the puncture test. Moreover, this technique permits the determination of the change in strength of thick rubber components which are subjected to different periods of ageing and different temperatures.

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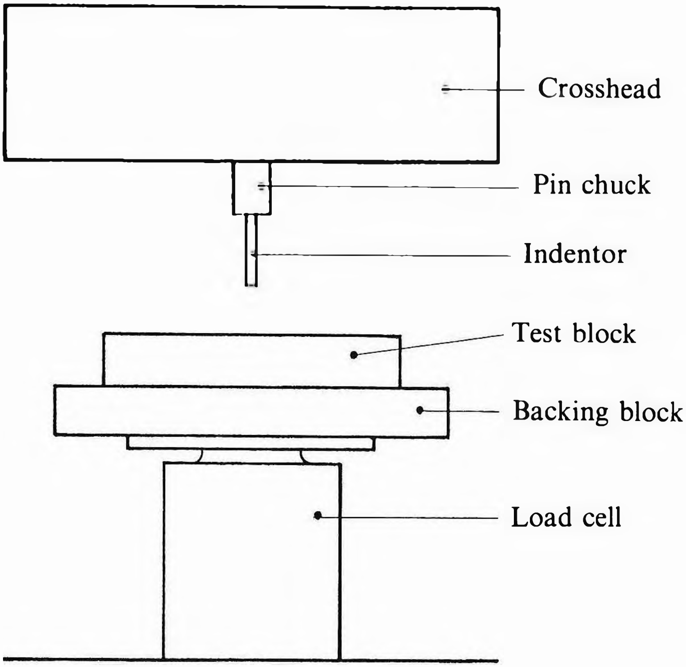

Figure 1. Schematic diagram of a puncture test.

In 1974, an engineering company provided Tun Abdul Razak Laboratory in Hertford, England, with a rubber sample from a ninety-six-year-old rubber bearing from a viaduct in Melbourne, Australia. This rubber was analysed and re-formulated to allow the ageing behaviour of thick rubber blocks to be studied at various times up to and including ninety-six years.

EXPERIMENTAL METHOD

The formulation of the ninety-six-year-old rubber was determined using thermogravimetric analysis (TGA) and thin layer chromatography (TLC) techniques[7]. The analyses suggest the following composition in parts per hundred of rubber (p.p.h.r.) by weight:

Natural rubber	100
Calcium carbonate	70
Ferric oxide	10
Sulphur	11

About 2.5 parts of other material, possibly dirt, were also present. The amount of free sulphur was less than 0.1%, indicating that the rubber was fully cured. The analysis also showed no evidence of curing residues or synthetic antioxidants. This is not surprising, because curing systems other than of sulphur plus metal oxide did not come into use until 1906, and synthetic antioxidants were not used until the 1940s.

A fresh natural rubber (RSS 3) compound was prepared using the above formulation and 5 parts of pine tar oil were added to the formulation to aid mixing. The rubber blocks were direct-compression-moulded for 3 h at 140°C to dimensions of 23 × 23 × 2.54 cm. The rubber blocks were then cut with a sharp knife into 2.5 cm test-cubes.

Ten-year-old samples of similar rubber prepared according to the above formulation by the late Dr Lindley of the Tun

Abdul Razak Laboratory, were also evaluated.

All the samples were aged for various times in an air-circulating oven at selected temperatures up to 200°C. One block was used for each combination of ageing time and temperature. After removal from the ovens, the blocks were allowed to cool at 23°C before being tested at room temperature. The blocks were cut open with a razor blade, and several puncture tests were carried out on both the exterior surface and the cut surfaces of the bulk rubber. Puncture tests were carried out using a 0.5 mm diameter indentor with 5 μm corner radius. Puncture tearing energies were subsequently obtained for each puncture test using the equation[1] $T_c = F(1-\lambda_c)/2\,\pi r_o$ where F is the puncture force at rupture, λ_c is compression ratio and r_o is the surface crack radius after puncture as shown in *Figure 2*. The determination of λ_c was discussed in Kamarudin[1] and it is equal to $\frac{1}{\lambda^2}$, where λ is given by:

$$\frac{\text{Radius of the indentor}}{\text{Radius of puncture crack } (r_o)}$$

It has been shown that oxidative degradation in rubber is accompanied by an increase in oxygen content[8]. Thus measurement of oxygen uptake provides a simple and quick way of evaluating the degree of degradation that has taken place in the rubber. Therefore, it would be of interest to find out the variation of oxygen content in the surface and in the bulk of the aged rubber blocks previously used for the determination of puncture energy. The instrument used was a Perkin-Elmer 240 Elemental Analyser which can give the value of the oxygen content to an accuracy of ± 0.3%. The method consisted of pyrolysing the sample in a stream of helium at 975°C over carbon, when the resulting oxygen was converted to carbon monoxide. The carbon monoxide was then passed through copper oxide at 670°C, where it was converted to carbon dioxide which was measured. The machine sub-

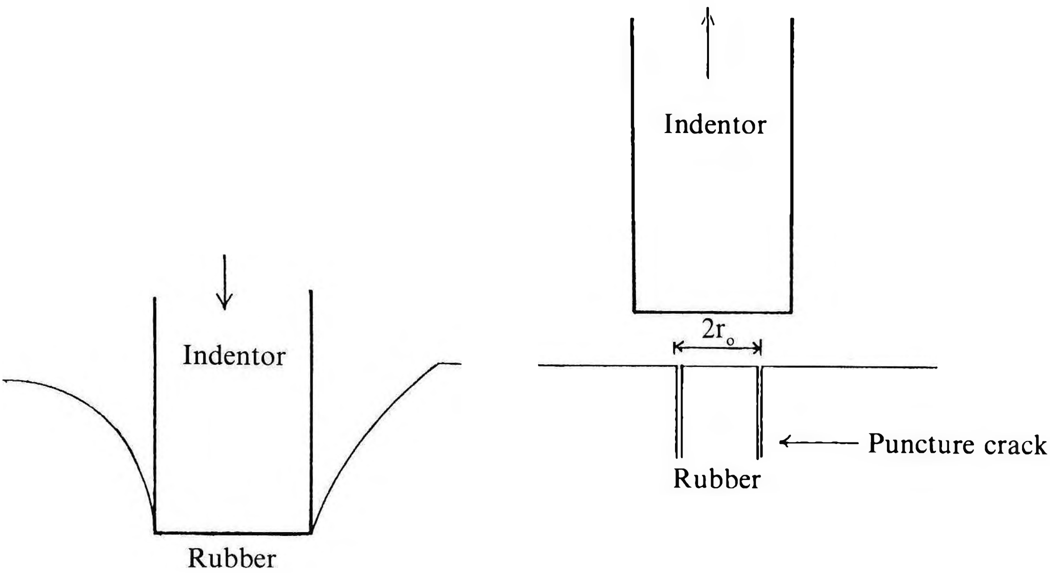

Figure 2. Schematic diagram of an indentation and puncture of rubber.

sequently gave the oxygen content in weight % (% w/w), *i.e.* as gramme of oxygen per 100 g of rubber vulcanisate.

RESULTS

Conditions of Natural Rubber Pad after Ninety-six Years' Service

The rubber pads were about 2.5 cm thick and *Figure 3* shows the locations of the pads between the steel structures and the supporting piers of the viaduct. *Figure 4* shows a clean-cut section through the sample to illustrate the depth of any ageing of the rubber. The surface of the rubber is hard and shows evidence of oxidation. Below a depth of about 1.5 mm, however, the rubber is free from such degradation and was there, found still to have a hardness of only 63 IRHD. There was no evidence of significant deterioration of the rubber below this depth and in the centre of the pad. This provides convincing evidence of the fact that the weathering of natural rubber in normal conditions is limited to the surface.

Variation of Puncture Energy with Time of Ageing

These tests were carried out on 2.5 cm cubes aged in air at 23°C, 40°C, 70°C, 100°C, 125°C, 150°C, 175°C and 200°C. The variations of puncture energy of the surface and bulk with time of ageing are shown in *Figures 5 – 12*. The puncture energy scales are linear and the time of ageing scale is logarithmic. *Figure 4* shows the results of air-ageing at 23°C. The ninety-six years' point was obtained from the original rubber from the Melbourne viaduct rubber pad. The puncture energies of the surface and bulk were similar within the first six months of ageing time. Then, the puncture energy dropped sharply. In the case of rubber at the surface, the puncture energy dropped sharply between six months and four years of ageing. With further ageing, it showed little change, but with the limited data available there is an indication of a minimum at about ten years with a subsequent small rise between ten and ninety-six years. In addition, the hardness (80 IRHD) of the surface of the ninety-six-

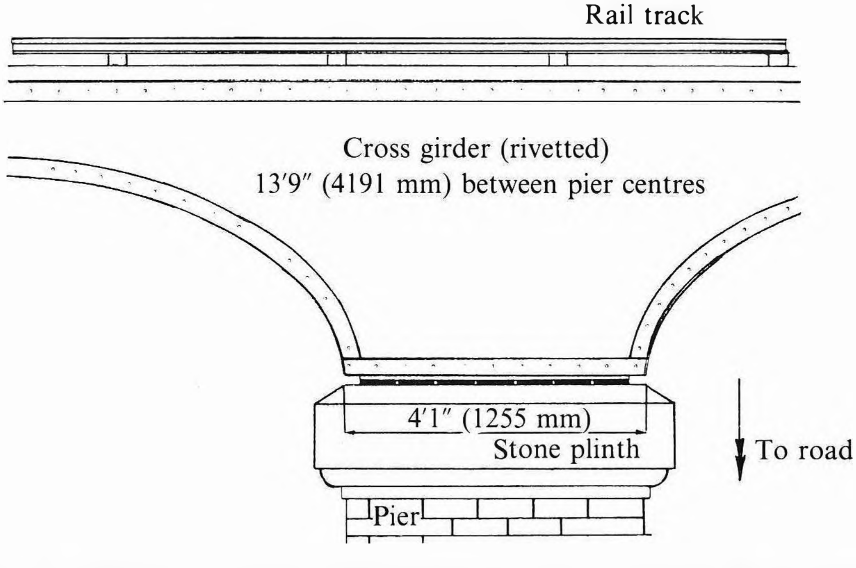

Figure 3. Drawing of Melbourne Railway Viaduct incorporating natural rubber pads in 1889 (traced from: N° 715, Raiway Department, Melbourne, May 1883).

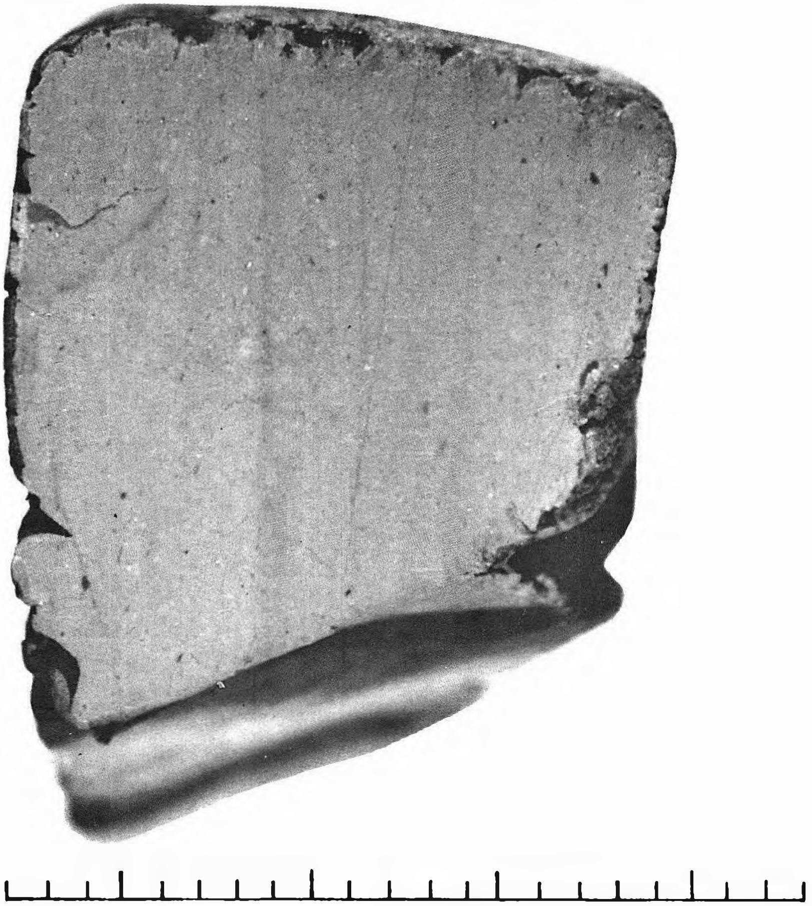

Figure 4. Cut section through ninety-six-year-old rubber pad showing that degradation was limited to an outer skin.

year-old rubber is higher than the hardness of the surface of the ten-year-old rubber (42 IRHD). In the case of the rubber in the bulk, the minimum puncture energy was not observed – the puncture energy obtained from the ninety-six-year-old sample was about 10.0 kJ/m.

For the rubber aged at 40°C (*Figure 6*), a minimum puncture energy was not observed for either the surface or the bulk. This is probably due to insufficient ageing time because a minimum puncture energy could be seen when the rubber was aged at 70°C *(Figure 7)*. In this case, the puncture energies of both the surface and the bulk showed minima. Moreover, at the minimum puncture energy, the surface of the rubber was soft, and with increasing ageing time, the surface became hard. The increase in puncture energy corresponded to the formation of a thin layer of hard skin as shown in *Figure 13*. This rubber was aged at 70°C for 181 days.

Figures 8 – 12 show the results of ageing at 100°C to 200°C. It can be seen that in all cases, there was no minimum puncture energy for both the surface and the bulk of the rubber. The puncture energy of the

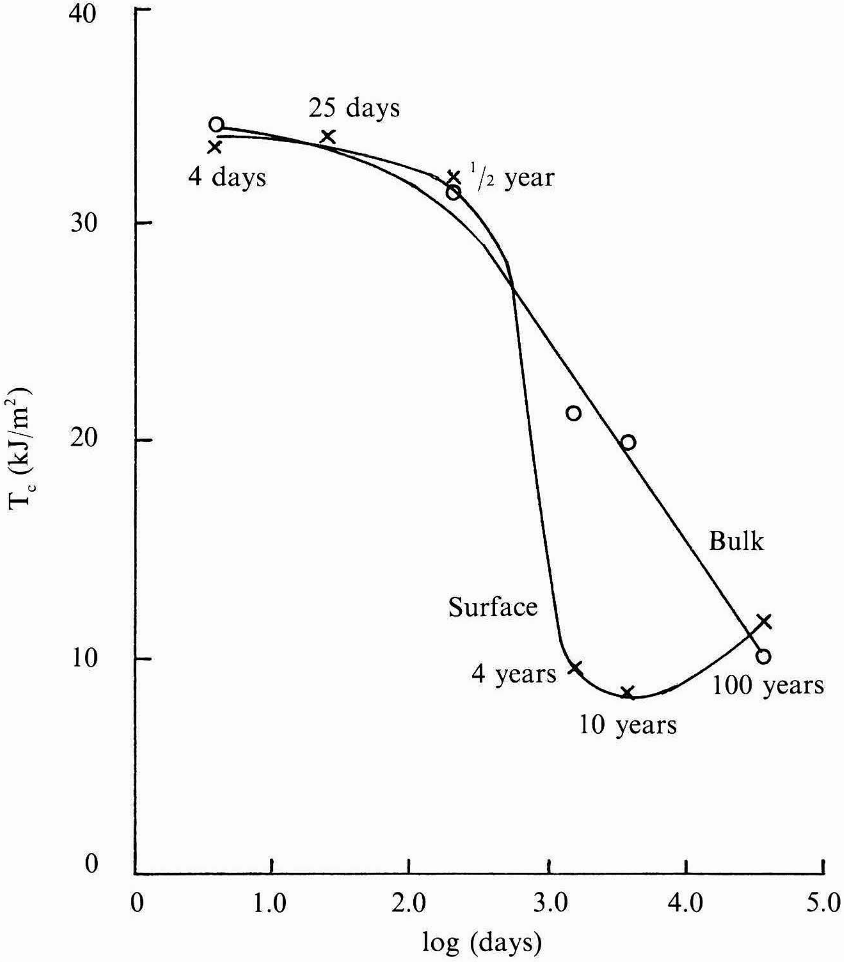

Figure 5. Variation of puncture energy of the surface and bulk of rubber with time of ageing in air at 23°C.

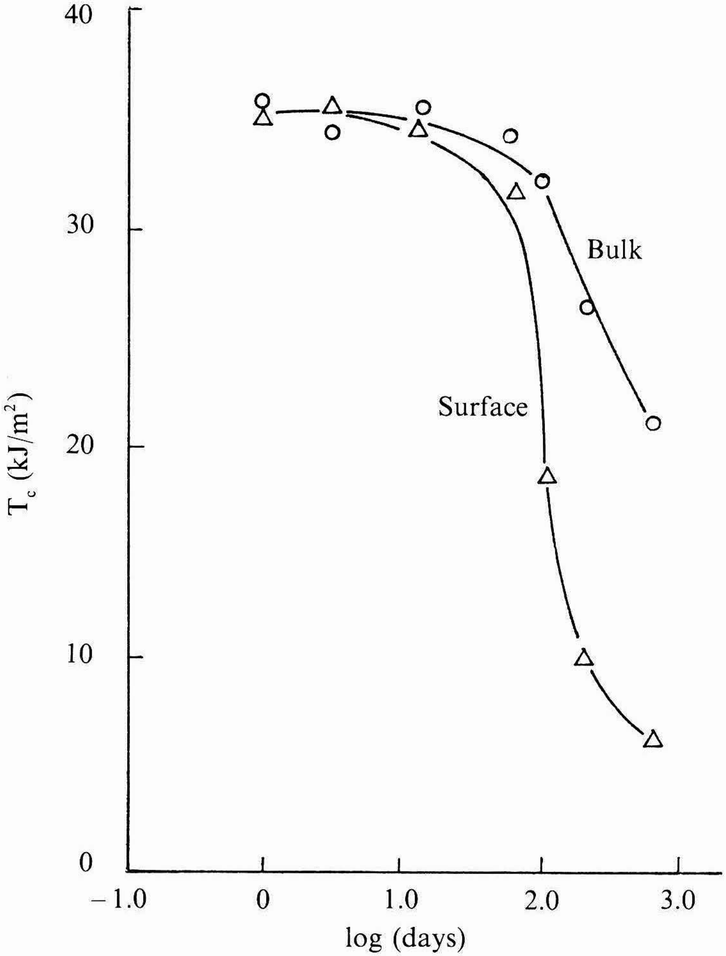

Figure 6. Variation of puncture energy of the surface and bulk of rubber with time of ageing at 40°C.

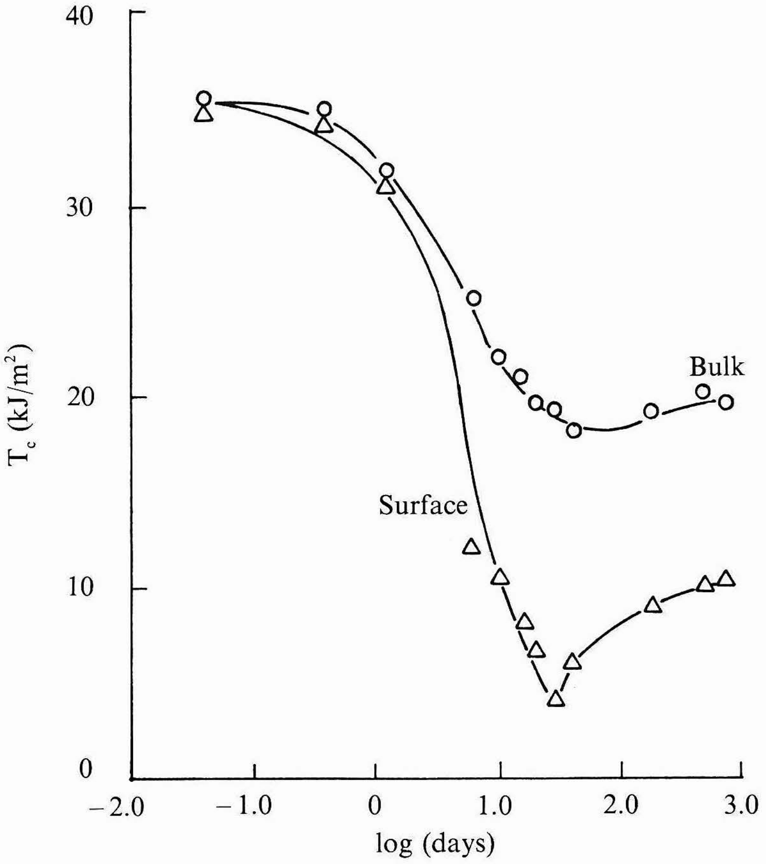

Figure 7. Variation of puncture energy of the surface and bulk of rubber with time of ageing at 70°C.

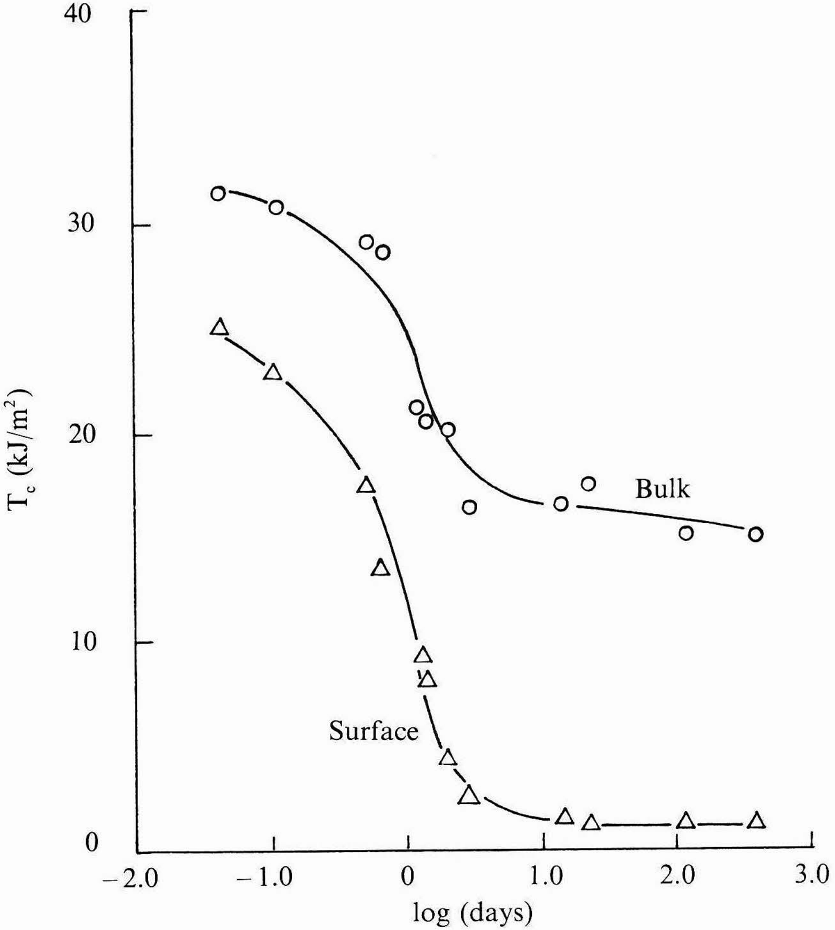

Figure 8. Variation of puncture energy of the surface and bulk of rubber with time of ageing at 100°C

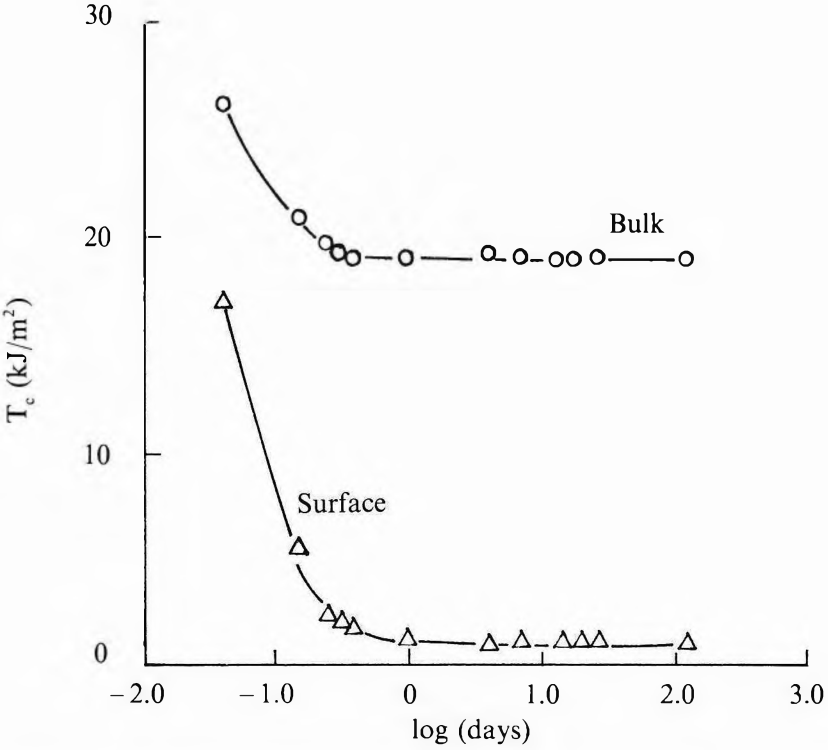

Figure 9. Variation of puncture energy of the surface and bulk of rubber with time of ageing at 125°C.

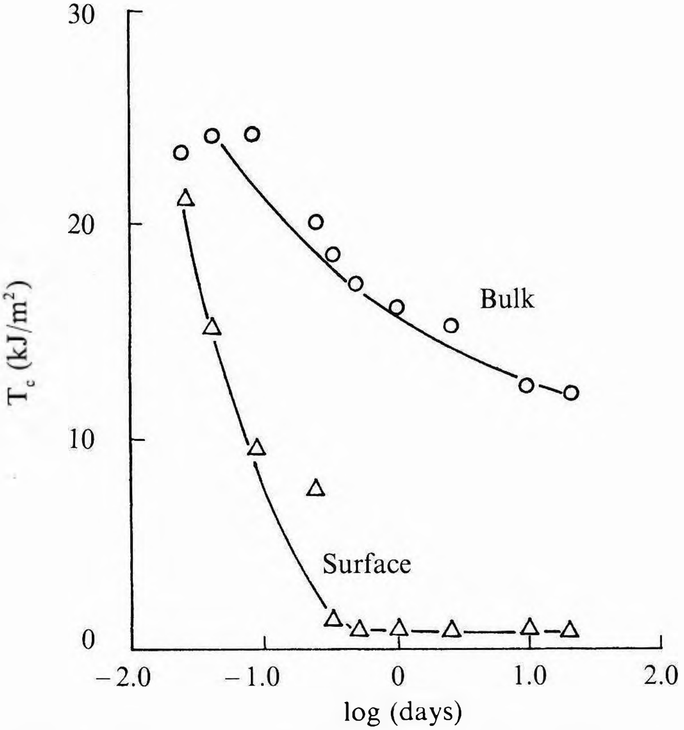

Figure 10. Variation of puncture energy of the surface and bulk of rubber with time of ageing in air ovens at 150°C.

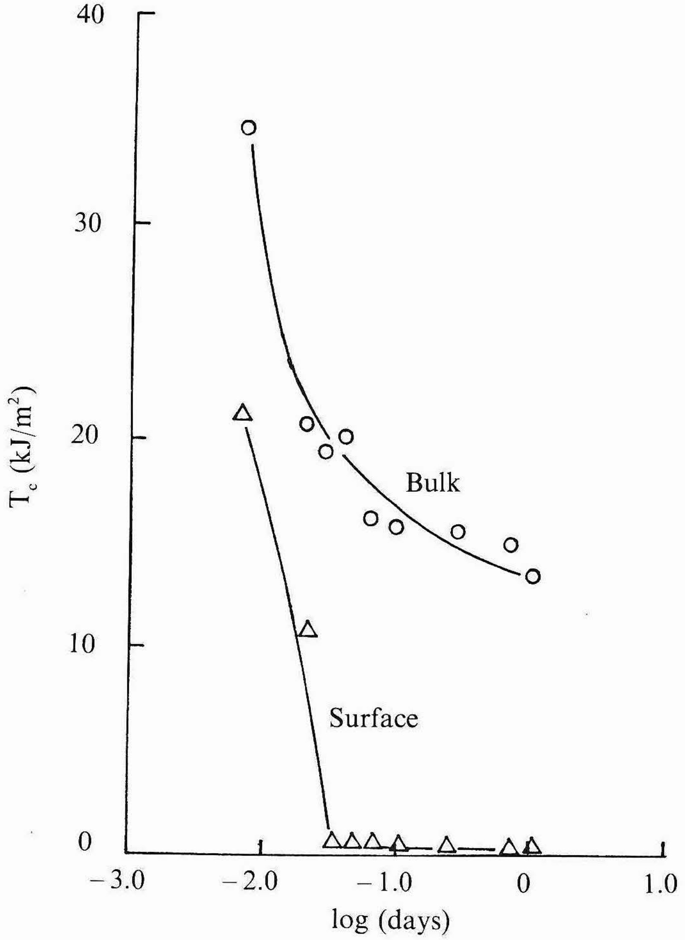

Figure 11. Variation of puncture energy of the surface and bulk of rubber with time of ageing in air oven at 175°C.

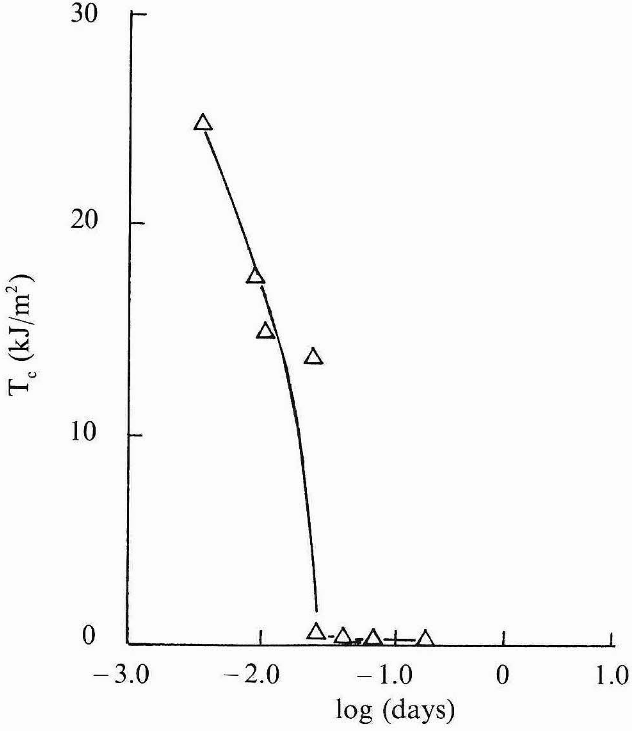

Figure 12. Variation of puncture energy of the surface of rubber ageing in an air oven at 200°C.

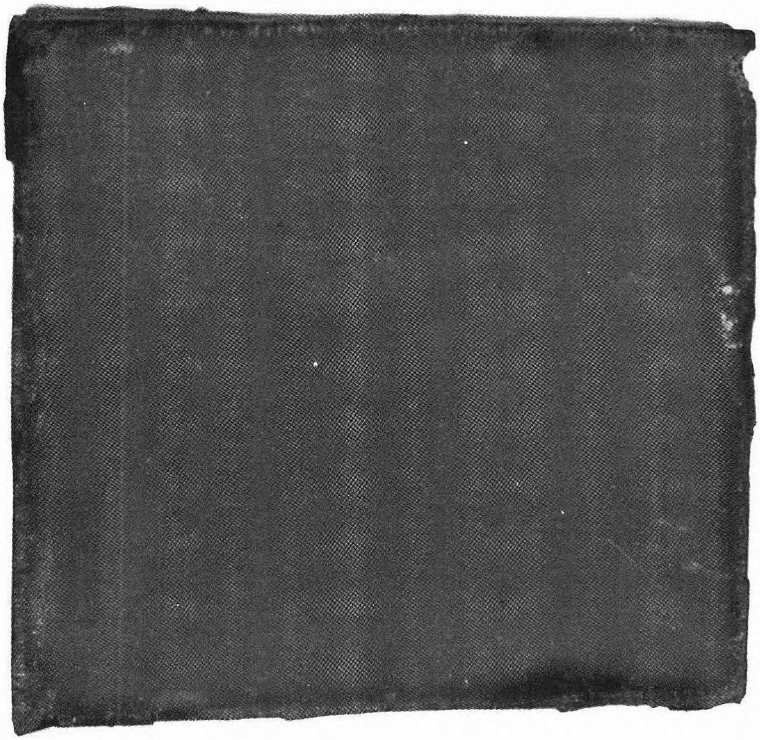

Figure 13. A hard skin on a rubber block aged at 70°C for 181 days.

surface rubber decreased with increasing ageing time, and at characteristic time t_a attained the lowest puncture energy value and remained at this value with increasing ageing times. The time t_a decreased with increasing temperature. In addition, the lowest puncture energy attained decreased with increasing temperature, for example, at 100°C and 200°C, the lowest puncture energies were 1.25 kJ/m^2 and 0.25 kJ/m^2 respectively. The formation of a thin skin could be observed after ageing time t_a, but the skin was soft and sticky at 175°C and 200°C.

The puncture energy in the bulk rubber aged at 100°C and 125°C was reduced to the lowest value and it appeared that the puncture energy did not subsequently vary with increasing ageing time. However, at 150°C and 175°C, the puncture energy showed a gradual reduction with increasing ageing time and did not show a constant value. The puncture energy could not be obtained with rubber blocks aged at 200°C because in the bulk of the rubber, there were small holes *(Figure 14)* which interfered with the puncture test. This provides an interesting example of the limitations of elevated temperature tests as accelerated ageing of room temperature behaviour.

Uptake of Oxygen

Figure 15 shows the variation with time of ageing at 23°C of the oxygen content of the surface and the bulk rubber. The results also include the oxygen content of the ninety-six-year-old rubber. It can be seen that at 23°C for rubbers prepared and aged up to four years, the bulk rubber did not increase in oxygen content. For the

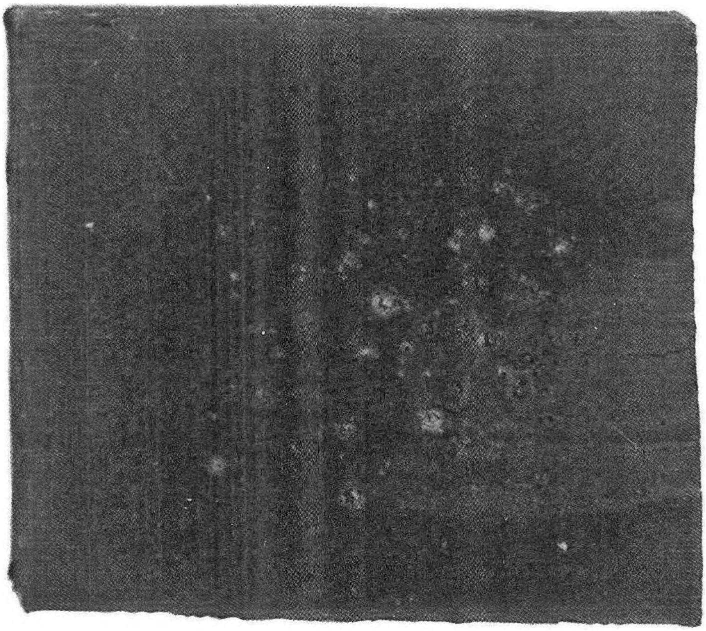

Figure 14. Holes in the rubber bulk aged at 200°C.

ten-year-old sample, the oxygen content of the bulk rubber was quite similar to that aged up to four years. The oxygen content in the bulk of the ninety-six-year-old sample was higher by about 4%. However, the increase in the oxygen content was more pronounced in the surface. The total oxygen content in the surface of the ninety-six-year-old sample was 10% higher than that in the four-year-old sample. *Figure 16* shows the increase in the oxygen content in the bulk of rubbers aged at different temperatures and times. Except for the 4% increase for the ninety-six-year-old rubber, the increases in the oxygen content in all other cases were less than 2%. The increase in the oxygen content was much higher in the surfaces of the rubbers, as shown in *Figure 17*. Most of the increase was about 10%. It can also be seen that the increase in the oxygen content was much quicker at higher temperatures than at 70°C or 23°C.

DISCUSSION

In general terms, puncture energy decreases with ageing. At very long ageing time near ambient temperature (as represented by the Melbourne bridge sample), there is some indications of a slight rise in puncture energy of the surface. Further evidence for a puncture energy minimum is obtained for ageing at 70°C in both the bulk and the skin. Measurements at 40°C may not have been continued long enough to reveal the minimum. At 100°C and above, the minimum is not observed. For the surface, the puncture energy decreases progressively to a very low level and then remains approximately constant.

A characteristic time t_a can be defined for each ageing temperature which represents the time to reach either the minimum in the puncture energy curve or the time to attain the low level of puncture energy

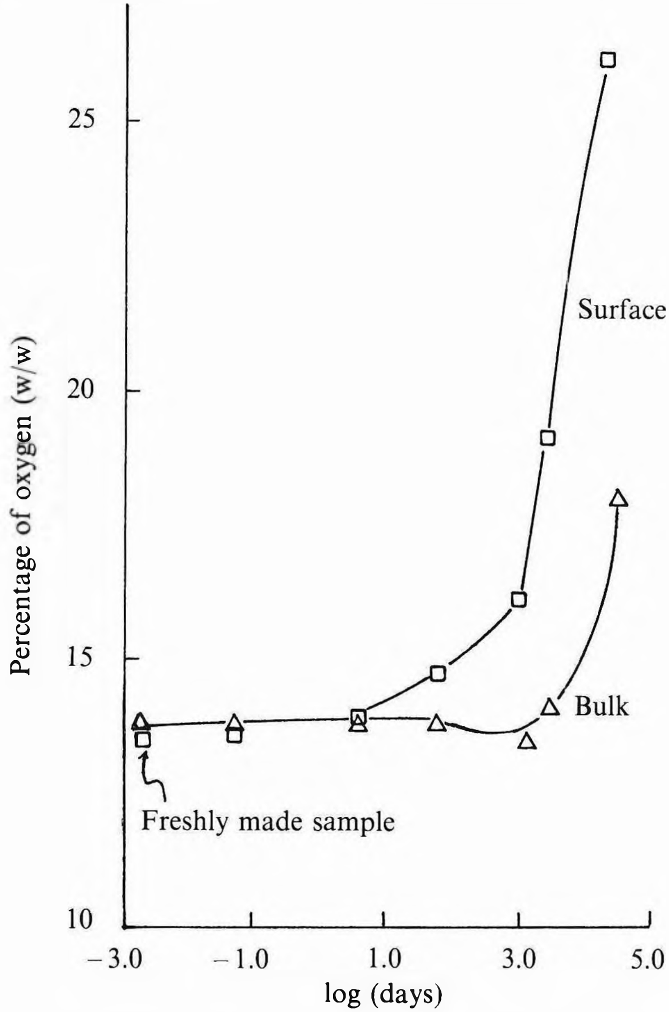

Figure 15. Variation of oxygen content with ageing time at 23°C.

characteristic of long-term ageing. At the higher temperatures (>70°C), this time has been taken as the time to the first puncture energy value which lies clearly with the group of nearly constant energies.

The time t_a, can be considered as a measure of the rate of the ageing process. A plot of log(t_a) *versus* reciprocal absolute temperature *(Figure 18)* is approximately linear and an Arrhenius activation energy[3] of 78.5 kJ/mole can be evaluated from its slope. This activation energy agrees well with a value of 80 kJ/mole reported by Lindley and Teo[3] for the chemical reaction relating to the skin formation during ageing of a modern accelerated efficient vulcanisate system. The network structure of the efficient vulcanising system consists of mainly monosulphidic crosslinks[9], whereas the vulcanisate studied here is based on metal oxide/sulphur system and therefore would give different crosslinking network structure. The two vulcanisate structures have different long-term maturing characteristics. The similarity in the

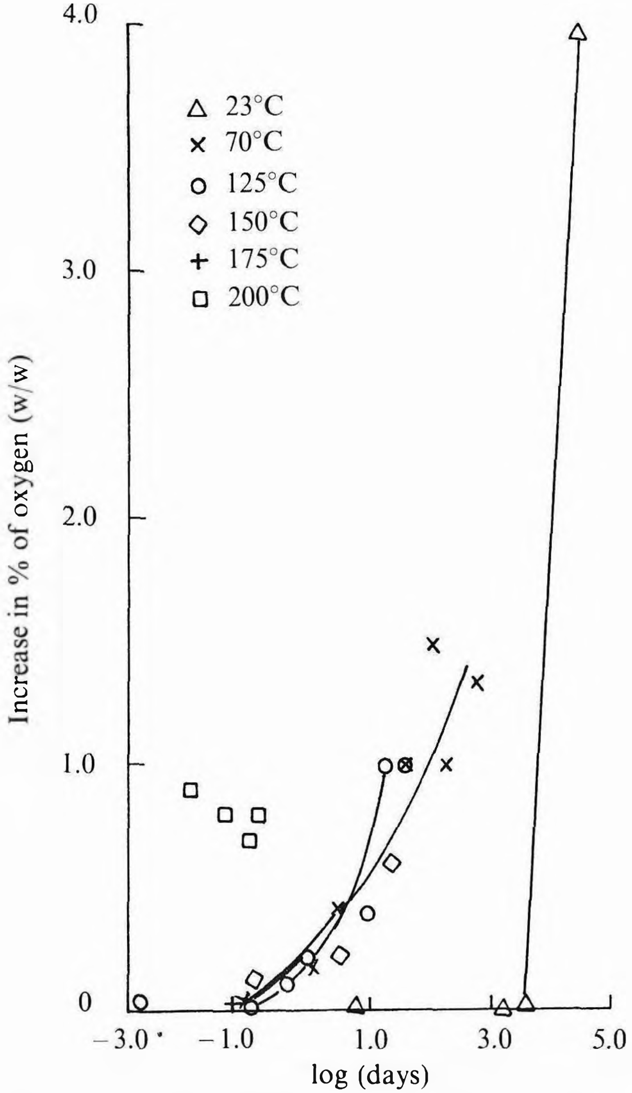

Figure 16. Increase in percentage of oxygen in the bulk of the rubber with ageing time.

activation energies for the ageing of the two materials suggests that this ageing is primarily the effect of oxidation rather than maturing reactions.

Evidence to oxidation is clearly provided by the change in the oxygen content of the samples. The change is more pronounced in the surface than in the bulk of the sample, and the pattern parallels the puncture energy data from surface and bulk measurements. The observations support the proposal made by Van Amerongen[10] and Stenberg *et al.*[11,12,13] that the surface skin acts as a protective barrier for the bulk of the sample. An important feature of the present results is that the effects of this surface skin can be detected even at quite modest ageing temperatures.

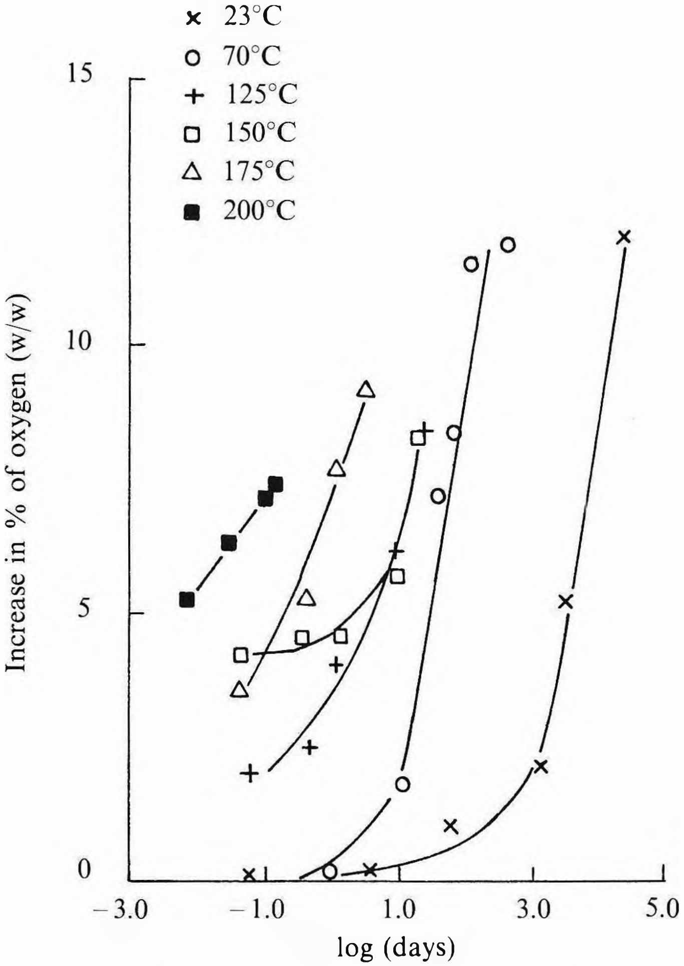

Figure 17. Increase in percentage of oxygen in the surface of the rubber with ageing time.

Puncture energy therefore bears a general relationship to oxidative ageing for the specific case of the metal oxide/sulphur vulcanisate system studied here. The study has taken specific advantage of the availability of the ninety-six-year-old sample which has been exposed to natural air ageing conditions and the results for this sample have been shown to be consistent with the shorter term, accelerated ageing results based on the newly prepared samples of the same formulation. Notwithstanding the fact that the formulation analysed may not be exactly the same 100 years ago, but nevertheless a technique has been developed here using the puncture test to characterise long-term ageing of thick rubber blocks.

The increase in the puncture energy after the minimum is obviously advantageous to rubber components, but the reason for the increase is not obvious. Two competing processes during ageing, namely chain scission and crosslinking, influence the puncture energy of the rubber.

Initially, the puncture energy is reduced due to oxidative chain scission as observed. With prolonged ageing, a stage will be reached where most of the rubber at the

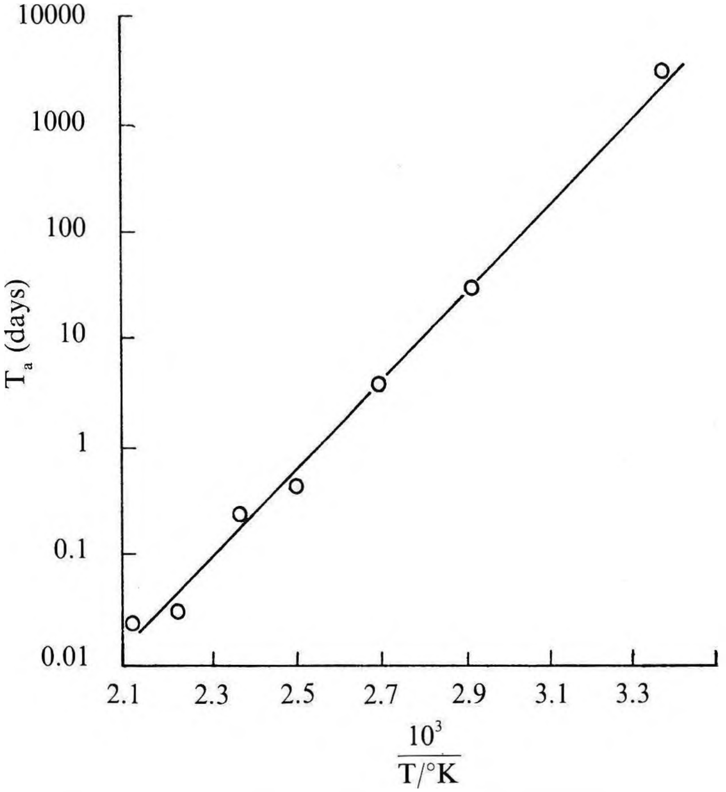

Figure 18. Arrhenius plot of the logarithm of the time t_a against reciprocal temperature.

surface has reacted with oxygen and forms an oxidised skin thus preventing further penetration of oxygen. In this situation, it is probable that the crosslinking becomes dominant. The rubber used in this investigation contained no accelerator ingredient to increase the rate of crosslinking and to increase the efficiency of utilisation of sulphur in forming the crosslinks. Thus, the initial degree of crosslinking formed after vulcanisation is low[14] even though 11 parts of sulphur per 100 parts of rubber were used. Smith *et al.*[15] showed that a slow crosslinking reaction takes place over long reaction times which gradually increase the number of crosslinks. However, the increase in the number of crosslinks will not be limited by the inefficiency of the rubber vulcanising system. It has been shown[16] that the strength of rubber passes through a maximum as the degree for crosslinking is increased. Therefore, the apparent increase in the puncture energy with increasing ageing time may be caused by a small increase in the number of crosslinks. A large increase in the number of crosslinks would have reduced the puncture energy.

However, the increase in the puncture energy after the initial drop was not observed when the rubbers were aged at 100°C or higher. This may be explained by the fact that at high temperatures, the crosslinks are thermally unstable[17]. At temperatures around 200°C, the main polyisoprene chains themselves becomes susceptible to decomposition[18]. At the surface, where oxygen is available, the situation is worse as the unstable crosslinks and the main chains can readily undergo oxidative ageing.

CONCLUSION

The puncture test was used to study the strength of thick rubber blocks which had been exposed to long-term ageing and high temperatures. A surface skin was observed on the ninety-six-year-old rubber pad which was found to be in good condition and still functioning. Such longevity is believed to be due to the formation of a hard surface skin, which is impervious to penetration by oxygen and thus protects the rubber underneath. A characteristic time t_a was obtained using a puncture test. Arrhenius plots of the characteristic times up to ten years and that of ninety-six years were shown to be linear. The activation energy was found to be 78.5 kJ/mole.

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Learning-by-doing in Production Function: The Case of the Malaysian Rubber Estate Sector

MUZAFAR SHAH HABIBULLAH*

The main objective of this study is to determine empirically the role of the learning-by-doing variable by employing the variable elasticity of substitution (VES) production function for the Malaysian rubber estate sector. Results obtained indicate that technical progress defined by learning-by-doing has played an important role in the development of the Malaysian rubber estate sector for the period under study. The results further suggest that the appropriate production function for the Malaysian rubber estate sector is the VES production function specification and capital deepening had positively affected the performance of the Malaysian rubber industry.

Use of the concept of learning-by-doing in economics was pioneered by Arrow[1] who stated, '... *knowledge is growing in time ... knowledge has to be acquired .... The acquisition of knowledge is what is usually termed "learning" .... Learning is the product of experience. Learning can only take place through the attempt to solve a problem and therefore only takes place during activity'.* Arrow[1] further advanced that, technical change in general can be ascribed to experience, and that, it is the very activity of production which gives rise to problems for which favourable responses are selected over time. Thus, there is a time lag between the introduction of new technology and the adoption or diffusion of new technology. The gap between the introduction and the adoption of new technologies is ascribed to the learning process.

In the agricultural sector, the process of learning is important for rapid adoption of new technologies to increase output. However, the rate of diffusion of new technology differs in different regions or countries, for example, Sri Lanka, Indonesia and Malaysia have different rates of technological diffusion. Diffusion of new technology in the Malaysian rubber sector has been rapid[2]. Comparing the smallholder sector and the estate sector, the latter shows a higher rate of diffusion of new technology[3]. Despite rapid diffusion of new technology in the rubber sector, the process of learning-by-doing has been noted by Yee and Longworth[4] who stated[†], *'The magnitude of these neutral upward shifts from HYM1 to HYM2 technology and from HYM2 to HYM3 technology for the 1964, 1970 and 1976 data sets are substantially lower than the shift from USM to HYM1 technology as estimated from 1964 data set ... One explanation for the increase is that it takes time for the estate managers to learn how best to take advantage of the superior embodied technology in the new technological strata (HYM2 and HYM3)'.* On another occasion, Barlow and Jayasuria[2] indicated that in the learning process, skill acquisition and capital accumulation would be increased. The experience accumulated then, will

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[†] USM denotes unselected seedling material; and HYM1, HYM2 and HYM3 denote high yielding material recommended for commercial plantings during three different time periods, 1964, 1970 and 1976.

reduce the risk and uncertainty involved in the investment of new technologies.

The main objective of this paper is to determine empirically the role of the learning-by-doing variable in the Malaysian rubber estate sector employing the variable elasticity of substitution (VES) production function. It has been recognised that the elasticity of substitution can be variable depending on the output and/or factor combinations. As the factor price ratio does not remain constant, changes will obviously vary the capital/labour ratio thus varying the elasticity of substitution. The VES production function assumes that the elasticity of substitution between capital and labour varies along the isoquant.

METHODOLOGY

Chew[5], Daud *et al.*[6] and Yee and Longworth[4] employed the Cobb-Douglas production function to the Malaysian rubber sector. However, according to Bairam[7,8,9], a more appropriate production function where learning-by-doing can be incorporated directly is the VES production function which has been popularly used in the literature because it includes the Cobb-Douglas production function as a special case[10,11,12]. Further, the elasticity of substitution is not constrained to a constant value, as it varies along the isoquant.

The VES production function used in this study is specified as:

$$Q_t = A_t e^{\Phi t} E_t^{\theta} K_t^{\alpha} L_t^{1-\alpha} e^{\mu (K/L)_t} \qquad \ldots 1$$

where A_t is the initial level of technology

E is the index of learning

Q, K, L are the levels of output, capital and labour respectively

Φ is the Hicks-neutral rate of exogenous technical progress

θ is the learning coefficient

α and $(1-\alpha)$ are the elasticities of output with respect to capital and labour respectively

μ is the substitution parameter

t is time.

VES can be derived as follows, and the marginal products are defined as:

$$\partial Q/\partial K = A_t e^{\Phi t} E_t^{\theta} (K/L)_t^{\alpha-1} e^{\mu (K/L)_t} [\alpha + \mu (K/L)_t] \qquad \ldots 2$$

$$\partial Q/\partial E = A_t e^{\Phi t} E_t^{\theta} (K/L)_t^{\alpha-1} e^{\mu (K/L)_t} [(1-\alpha) - \mu (K/L)_t] \qquad \ldots 3$$

Dividing *Equation 3* by *Equation 2* gives the marginal rate of substitution (MRS),

$$MRS_t = (K/L)_t \{[1/(\alpha + \mu (K/L)_t)] - 1\} \qquad \ldots 4$$

VES is then given by:

$$\sigma_t = \frac{d\,(K/L)_t}{d\,(MRS)_t} \cdot \frac{MRS_t}{(K/L)_t}$$

$$= 1 - \{\mu (K/L)_t /[(\alpha + \mu (K/L)_t^2 - \alpha]\} \qquad \ldots 5$$

Therefore, we can clearly observe that the elasticity of substitution is variable depending on the capital-labour ratio $(K/L)_t$ as shown by μ. As $\mu < O$, $\sigma < O$. If $\mu < O$, it is a labour-using situation where capital is substituted for labour. On the other hand, when $\mu > O$, capital deepening is experienced. However, it is clear from *Equation 5* that $\sigma < 1$, as $\mu < O$.

For the specification of variable learning-by-doing E, Arrow[1] suggested that E can be represented by cumulative output ΣQ, or cumulative investment ΣI. Arrow[1] pointed out that economic agents and technical changes are developed from experience gained within the production process. However, Arrow[1] favours the use of cumulative investment as an index of experience. He stated that cumulative investment as an index of learning provides more stimulant to innovation compared to, for example, cumulative output which was previously used. He also pointed out that a new machine produced and put into use is capable of changing the environment in which production takes place.

Using the same analogy, cumulative money balances ΣM, can also be used as an index of learning as cumulative money balances stimulate innovation[13]. Crouch[13] postulates that, '... *It is immediately apparent that monetaization, when viewed as a technological innovation, must lead unambiguously to higher steady-state growth values of income ... wage rate ... and the capital labour ratio...*'. Crouch's[13] argument is not without support. According to Moroney[14], it is useful to incorporate money in production theory as a source of technological change. He concluded that, '... *In this sense the invention of money might be viewed as a type of technological change both for firms and for the economy at large. ... In this simplest microeconomic context money should not be considered an ordinary input of the firm's production function. It is instead a source of technological change that may be regarded as an external economy for each firm*'. The fact is that, the important role of money in production has been empirically investigated by Sinai and Stokes[15] who provided the pioneering empirical work on this issue. Money balances act as a medium of exchange, facilitate the exchange between capital and labour for specialisation purposes and thus increase productivity. They also reduce the transaction cost and therefore, increase the economic efficiency of the money market system. More recently, Sarwar *et al.*[16] pointed out the important role of money in production. Money increases efficiency in obtaining inputs necessary for production and marketing of farm products. It facilitates the purchase of inputs in situation where the production process is lengthy and receipt of revenue is delayed.

On the other hand, McKinnon[17] pointed out that in developing countries subjected to fragmented capital markets and limited to self-finance, the accumulation of money balances can act as a conduit of investment. McKinnon[17] noted that, '*Suppose an individual saver-investor, being limited to self-finance, wants to purchase physical capital of a type that is different from his own output. He may store inventories of his own output for eventual sale when the capital assets are acquired, or he may steadily accumulate cash balances for the same purpose. The degree to which he relies on one mode or the other will depend on the real returns on holding money and the inconvenience of storing his own* product'. Thus, the greater the accumulation of money balances, the larger will be the inducement to invest. Therefore, money balances have a tendency to be positively related to the propensity to invest (theory and empirical evidence are detailed in Fry[18], Yoo[19] and Molho[20]).

Credit is a pre-requisite to diffusion of innovation[21,22], the process of innovation being endogenous. The ability of innovators to adopt new processes depends not only in the flow of new ideas and techniques available in the economy but most important of all to the availability of financial resources to undertake innovation. Schumpeter[22] stressed that, '*If anyone in an economic system in which the textile industry produces only with hand labour sees the possibility of funding a business which uses power-looms, feels equal to the task of overcoming all the innumerable difficulties, and has made the final decision, then he, first of all, needs purchasing power. He borrows it from the bank and creates his business*'. Thus, Schumpeter[22] showed that no innovation is possible without credit[23].

Barlow and Jayasuria[2] showed that due to the shortage of cash and non-existence of credit facilities in Indonesia, the rubber farmers were unable to adopt or take advantage of the new technologies. The important role of credit as an important factor to accelerate the adoption and diffusion of innovations has also been stressed by Dissanayake[24].

Based on the above literature, the following are alternative specifications for A_t:

$$A_t = A_o e^{\Phi t} E_t^{\theta} \qquad \ldots 6$$

$$A_t = A_o e^{\Phi t} \qquad \ldots 7$$

$$A_t = A_o E_t^{\theta} \qquad \ldots 8$$

where $E = \Sigma Q$, or $E = \Sigma H$, or $E = \Sigma I$, or $E = \Sigma M$, or $E = \Sigma RL$. Variable ΣH and ΣRL are cumulative hectarage planted and rubber loan respectively.. In agriculture, Q and H are highly correlated, so that Q can be represented by H.

For estimation purposes, *Equation 1* is constrained to linear homogeneity by dividing both sides by K, and after substituting *Equation 6* through the *Equation 8*, transformed into logarithm, the following equations are obtained:

$$\log (Q/K)_t = \log A_o + \Phi t + \theta \log E_t + (1-\alpha) \log (L/K)_t + \mu (K/L)_t + v_t \qquad \ldots 9$$

$$\log (Q/K)_t = \log A_o + \Phi t + (1-\alpha) \log (L/K)_t + \mu (K/L)_t + w_t \qquad \ldots 10$$

$$\log (Q/K)_t = \log A_o + \theta \log E_t + (1-\alpha) \log (L/K)_t + \mu (K/L)_t + \Gamma_t \qquad \ldots 11$$

Equation 9 proposed that technical progress is partly-exogenous and partly the result of learning-by-doing. *Equations 10* and *11* explain technical progress exclusively in terms of Hicks-neutral exogenous technical progress and learning-by-doing respectively. Further, if the estimated coefficient, μ is significant, then VES is an appropriate production function for the Malaysian rubber estate sector. Otherwise, the appropriate specification is the Cobb-Douglas production function.

Data and Method of Estimation

This study used annual time series data for the Malaysian rubber estate sector for the period 1965–86. Thus, the study is representative of the estate sector rather than the smallholder sector. Data for labour, capital, hectarage planted and output were compiled from various issues of *Rubber Statistics Handbook*[25] published by the Department of Statistics except for monetary data which was collected from various issues of *Quarterly Economic Bulletin*[26] published by Bank Negara Malaysia (see *Appendix* for detailed description of data). In this study, five versions of learning index were used, that is, cumulative output ΣQ, cumulative hectarage planted ΣH, cumulative investment ΣI, cumulative money balances ΣM, and cumulative rubber loan ΣRL. For money balances, we employed $M2$ ($\Sigma M2$) and $M3$ ($\Sigma M3$) definitions of money stocks.

The definition of capital is one of the controversial issues in studying production theory. As such, numerous definitions of capital have been given in the literature. Capital expenditure in agriculture is classified under modern *versus* traditional agriculture capital[27], durable *versus* non-durable capital[28], fixed *versus* variable capital[29], private *versus* public capital[30], *etc*. Nevertheless, the definition of capital expenditure in the border sense in the agricultural sector generally includes expenditure on land reclamation, land clearing, land improvements, irrigation, dams, farm building, dwellings, storage and warehouses, agricultural machinery and equipment (harvestors, threshors, ploughs, tractors, harrows), livestock, factor inputs (seeds, fertiliser, manure, insecticide, hired human labour), investment on research, education, skills and health for the development of human capital[31-37]. Therefore, in this study, the definition of capital expenditure includes expenditure on agriculture and plant machinery and equipment, transport equipment, new construction on residential dwellings, non-residential buildings, *etc*. The same definition of capital has been used by Habibullah[38].

All equations were estimated using ordinary least squares (OLS). However, when there was evidence of serial correlation, the maximum likelihood iterative estimation (MLE) technique of Beach and MacKinnon[39] to correct for serial correlation problem, was employed.

EMPIRICAL RESULTS

The regression results for the Malaysian rubber estate sector are presented in *Table 1*. Twenty-six equations were estimated by both OLS and MLE techniques. The estimated equations represented by *Equations 1a* and *1b* to *6a* and *6b* were equations where both definitions of technical progress; exogenous and learning-by-doing were incorporated. *Equations 7a* and *7b* incorporated only the exogenous technical progress variable, and *Equations 8a* and *8b* to *13a* and *13b* incorporated only the technical progress variable specified by learning-by-doing.

Results in *Table 1* show that the goodness of fit is satisfactory. However, regression results for ordinary least squares are not satisfactory because the Durbin-Watson (D.W.) statistics shows the presence of serial correlation. Furthermore, the coefficient (1–α) is greater than 1, signifying that the capital elasticity is negative which does not make economic sense[8,40]. As such, it was appropriate that the equations are estimated by the maximum likelihood estimation (MLE) procedure.

The results of MLE show that serial correlation is not present where D.W. statistics is about 2.0. However, when comparing all the equations in terms of the significance of all the parameters using t-statistics, we found that *Equations 8b* to *13b* are the appropriate specifications for log A, and therefore the most appropriate for the Malaysian rubber estate sector. We observed that all variables are significant at least at the 5% level. Furthermore, the coefficient (1–α) is less than 1.

The coefficient (1–α) which ranges from 0.90 to 0.97 implies that α ranges from 0.03 to 0.10. Thus, it suggests that the share of labour to total income during the period under study was 90% – 97% and that of capital ranges from 3% to 10%. The substitution parameters are all significant and positive. This implies that the VES production function is a more appropriate specification for the Malaysian rubber sector than the Cobb-Douglas production function. The parameters range from 0.08 to 0.13 depending on the different index of learning used. Positive values for μ imply that the variable elasticity of substitution (σ) between labour and capital are greater than unity. (The average value of σ using estimated *Equation 10b*, during the period under study, 1965 – 86, is 1.78.).

Our main interest is the importance of technical progress. Results in *Table 1* show that the learning variables are significant and positive. The learning parameter ranges from 0.07 to 0.18 depending on the different proxies of learning index used. The results suggest that learning-by-doing played a very important role in the development of the Malaysian rubber estate sector during the period under study.

This paper also presents several alternative measurements of learning index. This includes hectarage ΣH, money balance $\Sigma M2$ and $\Sigma M3$, and credit ΣRL. Our question is: Is there any significant difference between these alternatives compared to cumulative investment? To test this hypothesis, we employed the likelihood ratio test. *Table 2* shows the log-likelihood (L) value for each of the specification involving varieties of E. We observed that twice the difference in log-likelihood values for ΣQ, ΣH, $\Sigma M2$, $\Sigma M3$ and ΣRL [asymptotically distributed as chi-square (X^2) are 3.42, 3.71, 3.59, 3.64 and 1.82 respectively] are well within the critical value of the X^2 distribution at the 5% level of significance (critical value = 3.84). Therefore, we rejected the hypothesis that the alternative specifications ΣQ, ΣH, $\Sigma M2$, $\Sigma M3$ and ΣRL are not the appropriate proxies for learning index for the Malaysian rubber estate sector.

CONCLUSION

This study suggested that the VES production function specification is appropriate for the Malaysian rubber estate sector. The elasticity of substitution estimated was above unity and had a positive trend, which

TABLE 1. REGRESSION RESULTS FOR MALAYSIAN RUBBER ESTATE SECTOR

		$\log A_o$	Φ	θ	$(1-\alpha)$	μ	R^2	D.W.	rho
I. Technical progress is partly exogenous and partly the result of learning-by-doing									
A. Where $E = \Sigma Q$									
1a	OLS	–5.3662	0.72434×10^{-2}	0.14883	1.0204	0.12076	0.9935	2.348	
		(–13.086)***	(0.92346)	(2.9634)***	(13.744)***	(3.0093)***			
1b	MLE	–4.9114	0.62967×10^{-2}	0.09730	0.96789	0.11458	0.9935	2.028	–0.308
		(–7.9500)***	(0.80269)	(1.3095)	(11.845)***	(2.7409)**			
B. Where $E = \Sigma H$									
2a	OLS	–5.4969	0.74442×10^{-2}	0.16371	1.0129	0.11798	0.9934	2.344	
		(–11.958)***	(0.94568)	(2.9196)***	(13.782)***	(2.9515)***			
2b	MLE	–4.9732	0.66242×10^{-2}	0.10419	0.96085	0.11158	0.9938	2.032	–0.301
		(–7.0073)***	(0.83188)	(1.2248)	(11.889)***	(2.6740)**			
C. Where $E = \Sigma I$									
3a	OLS	–9.4220	–0.05787	0.93101	1.1505	0.13666	0.9951	2.7035	
		(–6.9599)***	(–2.7519)**	(3.8659)***	(13.871)***	(3.8981)***			
3b	MLE	–8.6037	–0.05148	0.79153	1.1058	0.14405	0.9961	2.029	–0.547
		(–6.4292)***	(–2.6581)**	(3.3558)**	(14.767)***	(5.3284)***			
D. Where $E = \Sigma M2$									
4a	OLS	–9.7592	–0.09837	0.62516	1.0201	0.10587	0.9938	2.242	
		(–5.5529)***	(–2.5932)**	(3.1767)***	(14.294)***	(2.8902)***			
4b	MLE	–7.7690	–0.06206	0.40696	0.96709	0.10275	0.9939	1.982	–0.277
		(–2.8380)***	(–1.0961)	(1.3368)	(12.101)***	(2.8433)***			
E. Where $E = \Sigma M3$									
5a	OLS	–9.6348	–0.10262	0.61185	1.0301	0.11203	0.9937	2.259	
		(–5.4420)***	(–2.5384)**	(3.0831)***	(13.857)***	(2.9493)***			
5b	MLE	–7.5755	–0.06259	0.38593	0.97059	0.10631	0.9939	1.989	–0.283
		(–2.8420)***	(–1.0685)	(1.3003)	(11.646)***	(2.7916)**			
F. Where $E = \Sigma RL$									
6a	OLS	–5.5783	–0.00537	0.23536	0.99982	0.11762	0.9928	2.449	
		(–9.8589)***	(–0.41211)	(2.3189)**	(13.022)***	(2.7718)**			
6b	MLE	–4.7723	0.00231	0.10413	0.93382	0.10546	0.9940	1.970	–0.325
		(–5.9869)***	(0.15079)	(0.74004)	(12.135)***	(2.5059)**			
II. Technical progress is wholly exogenous									
7a	OLS	–4.1838	0.02047		0.90197	0.06138	0.9897	1.803	
		(–36.323)***	(2.6042)**		(11.826)***	(1.4481)			
7b	MLE	–4.1153	0.01366		0.89090	0.07507	0.9933	2.081	–0.137
		(–45.178)***	(2.0758)		(14.853)***	(2.2924)***			
III. Technical progress is wholly the result of learning-by-doing									
A. Where $E = \Sigma Q$									
8a	OLS	–5.5167		0.17523	1.0101	0.12912	0.9931	2.436	
		(–14.728)***		(4.2634)***	(13.826)***	(3.3183)***			
8b	MLE	–5.1705		0.13374	0.97920	0.13080	0.9935	2.013	–0.371
		(–10.935)***		(2.5571)**	(13.052)***	(3.7920)***			

TABLE 1. REGRESSION RESULTS FOR MALAYSIAN RUBBER ESTATE SECTOR (CONTD)

	log A_o	Φ	θ	$(1-\alpha)$	μ	R^2	D.W.	rho
B. Where E = ΣH								
9a OLS	−5.67691		0.19378	1.00105	0.12614	0.9930	2.435	
	(−13.612)***		(4.2093)***	(13.871)***	(3.2424)***			
9b MLE	−5.2901		0.14762	0.97177	0.12830	0.9934	2.018	−0.367
	(−9.9074)***		(2.4874)**	(13.095)***	(3.7219)***			
C. Where E = ΣI								
10a OLS	−5.8857		0.29194	1.0102	0.09147	0.9927	2.036	
	(−11.634)***		(3.8538)***	(13.001)***	(2.4862)**			
10b MLE	−5.1833		0.18341	0.95104	0.09590	0.9946	2.044	−0.273
	(−11.766)***		(2.7494)**	(15.636)***	(3.5027)***			
D. Where E = ΣM2								
11a OLS	−5.3033		0.12187	0.94225	0.07125	0.9911	1.829	
	(−12.330)***		(3.2113)***	(12.491)***	(1.7925)			
11b MLE	−4.8078		0.07643	0.90783	0.08003	0.9935	2.053	−0.163
	(−12.445)***		(2.2147)**	(14.655)***	(2.5184)**			
E. Where E = ΣM3								
12a OLS	−5.2333		0.11436	0.94214	0.07197	0.9910	1.832	
	(−12.639)***		(3.1678)***	(12.376)***	(1.7992)			
12b MLE	−4.7647		0.07178	0.90811	0.08063	0.9934	2.056	−0.164
	(−12.924)**		(2.2045)**	(14.594)***	(2.5328)**			
F. Where E = ΣRL								
13a OLS	−5.4087		0.19956	0.99512	0.11076	0.9928	2.353	
	(−14.300)***		(3.9038)***	(13.460)***	(2.9140)***			
13b MLE	−4.8753		0.12347	0.94001	0.10986	0.9940	1.949	−0.346
	(−13.421)***		(2.4860)**	(15.382)***	(3.7848)***			

OLS and MLE denote ordinary least squares and maximum likelihood estimation, respectively.
*** Statistically significant at the 1% level.
** Statistically significant at the 5% level.
Figures within brackets are t-statistics.

Equation : $\log (Q/K)_t = \log A_o + \Phi t + \theta \log E_t + (1-\alpha) \log (L/K)_t + \mu (K/L)_t + \epsilon_t$

TABLE 2. LOG-LIKELIHOOD RATIOS (L)

Equation no.	Learning-by-doing variable	L
8b	ΣQ	29.11
9b	ΣH	28.97
10b	ΣI	30.83
11b	ΣM2	29.03
12b	ΣM3	29.00
13b	ΣRL	29.91

implies that capital deepening had a positive effect on the performance of the Malaysian rubber industry during the period under study. Further, this study has indicated that learning-by-doing has played an important role in the development of the Malaysian rubber estate sector. Several alternatives of learning-by-doing variables were tested and the results indicate that the model estimated can explain Malaysian rubber estate data quite well. However, the results are only preliminary and subject to further research.

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APPENDIX

Descriptions on Data Variables

Q = Total production in estate

L = Number of contract/hired labour in the estate

K = Capital expenditure of agricultural and plant machinery and equipment, transport equipment, new construction on residential dwellings, non-residential buildings (e.g. factories, offices, *etc.*)

ΣQ = Cumulative total production in the estate

ΣH = Cumulative total hectarage planted in the estate

ΣI = Cumulative total capital expenditure in the estate

$\Sigma M2$ = Cumulative money stock $M2$. $M2$ includes currency in circulation, demand deposits held by non-bank private sector, savings and fixed deposits held at commercial banks.

$\Sigma M3$ = Cumulative money stock $M3$. $M3$ equals $M2$ plus savings and fixed deposits held at National Savings Bank, finance companies and merchant banks.

ΣRL = Cumulative rubber loan outstanding at commercial banks

t = Time period, 1965 – 86

J. nat. Rubb. Res., **7(2)**, 152 – 155

SHORT COMMUNICATION

FTIR Studies on Amino Groups in Purified Hevea *Rubber*

ENG AIK-HWEE*, YASUYUKI TANAKA* AND GAN SENG-NEON**

FTIR spectrum of natural rubber shows characteristic bands of attached nitrogeneous compounds at 3280 cm^{-1} and 1540 cm^{-1}. These bands diminish if the fresh field latex is treated with enzyme followed by washing in the presence of surfactant; a band at 3316 cm^{-1} is noticeable after the treatment. The band at 3320 cm^{-1} that remains after the treatments suggests the presence of residual amino acids bonded to the rubber molecule.

Natural rubber contains various types of naturally occurring non-rubber materials, which are either tenaciously held or bonded to the main-chain molecule. While studies on the non-rubber constituents in latex have been well documented, the bonded non-rubbers on the polymer chain have attracted less attention. This is probably due to their presence at very low concentration level and consequently the difficulty in characterising them.

Proteins and amino acids in natural rubber have been thought to be physically held by the polymer. Although they have been assumed to be responsible for the branching and crosslinking of natural rubber[1], their direct bonding to the polymer has not been demonstrated.

This paper reports a new finding on the presence of residual amino groups in highly purified *Hevea* rubber.

MATERIALS AND METHODS

Alcalase 2.0 *M* was obtained from Novo Industries, Japan and latex concentrate, 60.2% d.r.c., from Soctex, Malaysia.

Method 1

15 ml of latex concentrate was diluted to 200 ml by distilled water and stabilised with 0.12% w/v sodium naphtenate. The pH was adjusted to 9.2 by the addition of sodium dihydrogen phosphate. 0.78 g of alcalase 2.0 *M*, dispersed in 10 ml of distilled water, was added to the diluted latex. The pH of the solution was again adjusted to 9.2 accordingly. The mixture was allowed to stand for 24 h at 37°C.

Procedure A. The reacted latex was coagulated by the addition of 2% v/v phosphoric acid and washed extensively with distilled water. The rubber was dried under vacuum and extracted with acetone for 16 h. It was then redissolved in toluene at 1% w/v, and centrifuged for 30 min at 11 000 r.p.m. The clear solution was separated and the rubber precipitated into excess methanol. The rubber was dried under vacuum at room temperature[2]. The control sample was prepared by repeating the same procedure, omitting the alcalase treatment.

Procedure B. Upon completion of the enzymatic treatment, 1% w/v Triton X-100

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was added into the mixture and centrifuged for 30 min at 11 000 r.p.m. The cream fraction was redispersed into 200 ml of distilled water containing 1% w/v Triton X-100 and recentrifuged for 30 min. The process was repeated three times and the rubber was isolated by the addition of calcium chloride. Centrifugation may be necessary to assist coagulation. The coagulated rubber was treated as described above.

Procedure C. Further purification of deproteinised natural rubber was performed with ethanolic hydrochloric acid. Thus 0.5 g of rubber obtained from *Procedure B* above was dissolved in 100 ml of nitrogen-purged toluene containing 0.5 ml acetic acid for 24 h in the dark. Approximately 50 ml, 1 *M* nitrogen-purged HC1 containing 10% ethanol was added into the solution and shaken for 20 min in a separating funnel. The rubber solution was separated by means of centrifugation and the isolated rubber solution was extracted with fresh ethanolic HC1. This was repeated four times. Finally, the excess acid was removed by washing with nitrogen-purged distilled water. The rubber was isolated by precipitating it into excess methanol. It was further purified by reprecipitation and dried under high vacuum.

Method 2

Latex concentrate was diluted 15 times with distilled water and stabilised with 1% w/v Triton X-100. The latex was centrifuged for 30 min at 11 000 r.p.m. and the cream was dispersed in a same volume of distilled water containing 1% w/v Trition X-100 and the centrifugation process was repeated. The procedure was repeated three times. The rubber was isolated with calcium chloride and extracted with acetone as described earlier.

Nitrogen contents in the *Hevea* rubber samples were analysed according to the procedure described in the RRIM test method[3]. Natural rubber was cast on KBr disk and transmittance was measured by a JASCO 5300 fourier transform infrared spectrometer for 300 scans with auto gain.

RESULTS AND DISCUSSION

Table 1 shows the nitrogen content of control sample which reflects the normal level of proteineous compounds in commercial latex concentrate. Purification by *Method 1, Procedure B* and *Method 1, Procedure C* gives much lower nitrogen content as compared to *Method I, Procedure A*. This is not totally suprising as in *Procedure A* the cleaved protein can be co-precipitated with natural rubber by phosphoric acid. Subsequent centrifugation after the deproteinisation process reduces nitrogen content to 0.01%. Alcoholic acid treatment as outlined, further reduced the nitrogen content to 0.009%.

FTIR study has revealed that non-purified rubber shows weak transmittance

TABLE 1. NITROGEN CONTENT OF PURIFIED NATURAL RUBBER

Sample	Nitrogen (%)	FTIR band (cm^{-1})
Control	0.16	3280, 1540
Method 1, Procedure A	0.05	3316, 3280, 1540
Method 1, Procedure B	0.01	3320
Method 1, Procedure C	0.009	3320
Method 2	0.04	3316, 3280, 1540

bands at 3280 cm^{-1} and 1540 cm^{-1} which are characteristic vibrations of >N–H and >N–C=0 respectively[4]. Upon successive surfactant washing, or enzymatic deproteinsation followed by acid coagulation, the intensities of these bands were significantly reduced. Concurrently, a band around 3316 cm^{-1} was found to be present. Further

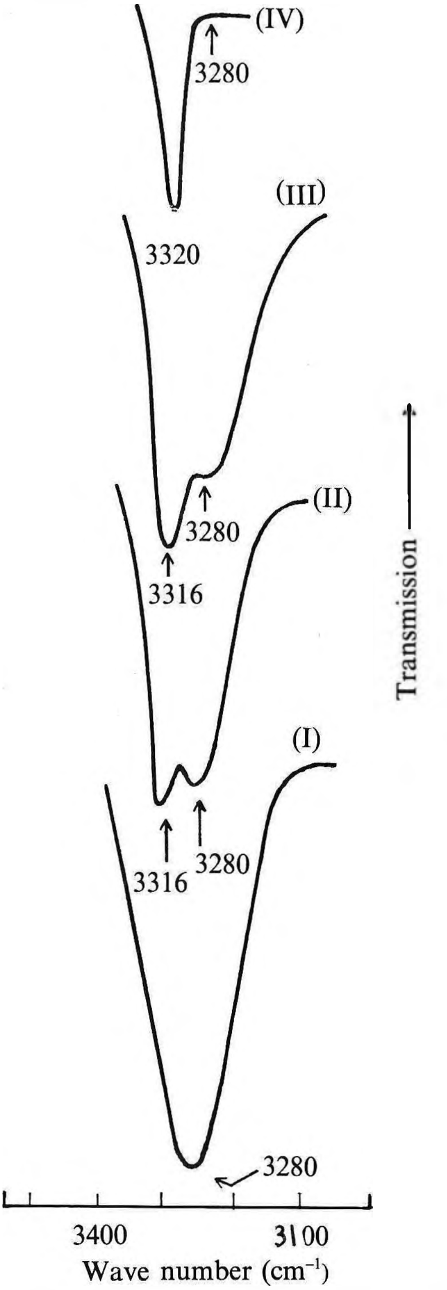

Figure 1. FTIR Spectra of purified Hevea *rubber.*

(I) Control, (II) Method 1, Procedure A, (III) Method 2, (IV) Methods 1, Procedure B and 1, Procedure C.

purification *via* successive surfactant washing after enzymatic treatment shows no detectable transmittance at 3280 cm^{-1} and 1540 cm^{-1}. The band at 3320 cm^{-1}, however, remained present even after acid extraction as shown in *Figure 1*.

Recent vibrational spectroscopic studies on a series of short chain peptides by Naumann *et al.*[5] showed that $>$N–H in terminal amino acid of peptide, demonstrated by dipeptide and tripeptide, has a vibrational frequency at 3315 – 3320 cm^{-1}. As the number of repeating units of peptide increases, the relative intensity of the inner $>$N–H units of the peptide linkages which have a vibrating frequency at 3280 cm^{-1}, increases while that of the terminal units reduce accordingly, as shown by tetrapeptide and pentapeptide. In addition, in the associated state (bonded) and dissociated state, the $>$N–H peaks appeared at about 3313 cm^{-1} and 3390 cm^{-1} respectively[6]. Therefore, the presence of $>$N–H band at 3320 cm^{-1} in purified natural rubber even after alcoholic acid treatment suggests that some proteins or amino acids are bonded to the natural rubber molecule. This is not totally unexpected since proteins and amino acids have been demonstrated to react with the abnormal groups of natural rubber giving rise to the storage hardening phenomenon of natural rubber[7,8].

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