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Branching in Natural Rubber

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The number of branch-points in natural rubber (NR) was analysed for the rubber from deproteinised fresh field (FL) and high-ammonia (HA) latices, using ^{13}C-NMR and osmometric measurement before and after transesterification of the rubber. About two branch-points per chain was found for the rubber from FL-latex and two to five by increasing M_w in proportion to that of HA-latex. The Huggins 'k' constants of the fractionated transesterified rubbers were lower than that of the original rubbers. The M_w and M_n values of each fraction decreased by about 10% - 50% after transesterification. The gel content of rubber from HA-latex was decreased from 55% to 15% after deproteinisation and further decreased to 1% after transesterification. The most part of gel in NR was presumed to be formed from some functional groups in NR by aggregation reaction with proteins. The branch-points are originated from other functional groups in NR, presumably such as phospholipids.

It is well recognised that a proportion of any commercial unmilled *Hevea* rubber is insoluble in rubber solvents, this portion being termed the gel phase[1]. Much work on the properties of this phase were done over several decades ago. The gel content varied with the source and type of rubber and depended on the nature of the solvent. If the gel phase is a simple crosslinked network, it should be insoluble in all the good solvents. The gel phase which is partially soluble in some solvents is also sometimes termed as soft-gel and recognised as one that cannot be a simple crosslinked network, but must have a more complex structure[2]. The gel component in synthetic *cis*-1,4-polyisoprene is certainly crosslinked rubber, arising from side-reactions during polymerisation and does not possess a phase such as that which is present in NR[3].

Rubber from fresh field latex (FL-latex) is normally completely soluble in rubber solvents, provided that the tree is regularly tapped[1,2]. However, commercially available high-ammonia latex (HA-latex) contains a lot of the gel phase. This demonstrates that some degree of crosslinking might have occured in HA-latex after or during storage. It has been hitherto believed that branching in NR originated from the abnormal groups such as aldehyde[4], epoxide[5] and lactone[6] groups. However, we have elucidated that these abnormal groups in NR are not major factors for branching and gel formations[7]. The branch-points in NR are classified into two types: (1) branchings due to proteins, which are easily broken down by enzymatic deproteinisation; and (2) branchings originated from long-chain fatty acids and/or phospholipids, which can be decomposed by transesterification. In the present work, we applied these two treatments to elucidate the behaviour of branching in NR by a combination of NMR, GPC and viscometric techniques.

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EXPERIMENTAL

Fresh field latex of RRIM 600 clone and commercial high-ammonia latex (HA) were used. The latex was diluted with distilled water to 15%–20% DRC, then adjusted to pH 9.2 before using with NaH_2PO_4, and treated with 0.04% (w/v) *Alcalase 2.0T*, in the presence of 1% (w/v) sodium dodecyl sulphate (SDS) at 37°C for 24 h, followed by centrifugation. The cream rubber was redispersed in 1% (w/v) SDS to make 15%–20% DRC and recentrifuged twice.

The deproteinised rubber (DPNR) was subjected to solvent fractionation in the usual way. The rubber fractions having a narrow polydispersity in molecular weight distribution were chosen for this study. A half portion of each fractionated rubber was subjected to transesterification in toluene as reported in the previous paper[7].

The gel-permeation chromatography (GPC) analysis was carried out on a set of two 60-cm columns packed with polystyrene-divinylbenzene copolymers, having exclusion limits of 2.0×10^7 and 5.0×10^4. Measurements were made using tetrahydrofuran (THF) at a flow rate of 0.5 ml/min at 30°C, monitoring with low-angle laser-light scattering (LALLS) and RI detectors. Commercially obtained standard polystyrenes were used for calibration. Samples were prepared at a concentration of 0.01% (g/dl) in THF at room temperature in the dark and were filtered through a Millipore LS prefilter and a 0.2 μm membrane filter before injection.

Viscometric measurements were performed in a single-bulb Ubbelohde viscometer. Measurements were made in a constant-temperature bath controlled at 30 ± 0.01°C and were repeated until three consecutive readings differed by ± 0.01 sec. All of solvents and the rubber solutions were filtered through a glass filter 2G-4 before measurements. An aliquot of the solutions was dried to constant weight for the determination of concentration. The intrinsic viscosity was determined by extrapolating the data to infinite dilution as given in *Equation 1*[8] and the Huggins' constant (k') was calculated using *Equation 2*:

$$[\eta] = 4.75 \times 10^{-5} M_v^{0.83} \quad \ldots 1$$

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad \ldots 2$$

where η_{sp}/c, $[\eta]$, c, M_v and k' represent the reduced viscosity, intrinsic viscosity, concentration expressed as g/dl, viscosity-average molecular weight and Huggins' constant, respectively.

The ^{13}C-NMR measurements were taken with a JEOL LA-500 spectrometer at 50°C, in deuterated chloroform. Absolute M_ns were obtained with a Wescan RC-51 membrane osmometer, at 35°C in filtered toluene solutions. The gel content was determined as reported in the previous paper[9].

RESULTS AND DISCUSSION

The gel content of rubber from FL-latex was reduced from 5% to 2% and 0.5% after deproteinisation and transesterification, respectively, and that of the rubber from HA-latex decreased from 55% to 15% and 1%, respectively. This suggests that gel fraction is abundant in the rubber from HA-latex rather than in FL-latex. It is presumed that certain reactions for gel formation proceed during the preservation of NR latex in the presence of ammonia. It is interesting to note that the gel

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content in the rubber from deproteinised HA-latex was reduced further from 15% to 4%, by re-proteinisation. Therefore, it is reasonable to presume that the oligopeptides, which have been confirmed to be residing even after extensive deproteinisation[10], would act as active functional groups for re-crosslinking in the presence of ammonia for the deproteinised latex.

Figure 1 shows a typical change in the molecular weight distribution (MWD) of rubber fractions from FL-latex after transesterification. A skewed unimodal MWD of the early precipitated fractions, *i.e.* the higher M_w fractions, became the bimodal distribution and showed a marked increase in the low M_w peak after transesterification. On the other hand, the low M_w fractions showed an unimodal distribution and the peak position was slightly shifted to the lower M_w direction after transesterification. The rubber fractions No. 6 to No. 10 showed an appreciable shift in the MWD, indicating that the middle M_w fractions contain a lot of branch-points.

The weight-average molecular weight ($\overline{M}_w$) and number-average molecular weight ($\overline{M}_n$) of each fraction are given in *Table 1*. After transesterification, the $\overline{M}_w$ and $\overline{M}_n$ values decreased by about 10% to 50% from the original values. The $\overline{M}_n$ values measured by osmometry also showed a similar tendency. The reduction of the $\overline{M}_w$ and $\overline{M}_n$ of these rubber fractions after transesterification was confirmed not due to the degradation of the main-chain of rubber, but the decomposition of branch-points to form linear molecules[11,12].

The intrinsic viscosities [η] of each rubber fractions before and after transesterification are listed in *Table 1*. The [η] value of the higher M_w fractions did not show much difference, but it decreased to about one-half for an extreme case in the lower M_w fraction. It is well-known that the Huggins' *k'* constant is a qualitative indicator of the presence of long-chain branching and for a given polymer, the *k'* is nearly independent of molecular weight and MWD and it increases in proportion to the quantity of branching in the polymer chains[13,14]. *Figures 2a* and *b* show the relationship between the M_w from GPC-LALLS measurement against the Huggins' *k'* constant for the deproteinised rubbers from FL- and HA-latices, before and after transesterification, respectively. It is obvious that the *k'* values of deproteinised rubber fractions after transesterification were lower than those of untreated ones in both cases. The *k'* values of the deproteinised rubber fractions from HA-latex were higher than those of FL-latex. This implies that the number of the long-chain branchings in the rubber from HA-latex is higher than that from FL-latex. The *k'* values of the deproteinised rubbers after transesterification were independent of molecular weight of the rubber fractions. This is probably due to the fact that all the transesterified rubbers are composed of linear molecules. It was also observed that the low M_w fractions showed similar *k'* values before and after transesterification. This also suggests that the low M_w fractions consisted predominantly of linear rubber molecules. It seems likely that if the low M_w rubber fractions contained one branch-point by fatty acid groups at the terminal side of rubber molecule[12], it should be treated as a linear polymer. This could result in an one-half reduction in the $\overline{M}_w$ value after transesterification.

The number of branch-points in polymer can be commonly determined by comparing M_w of

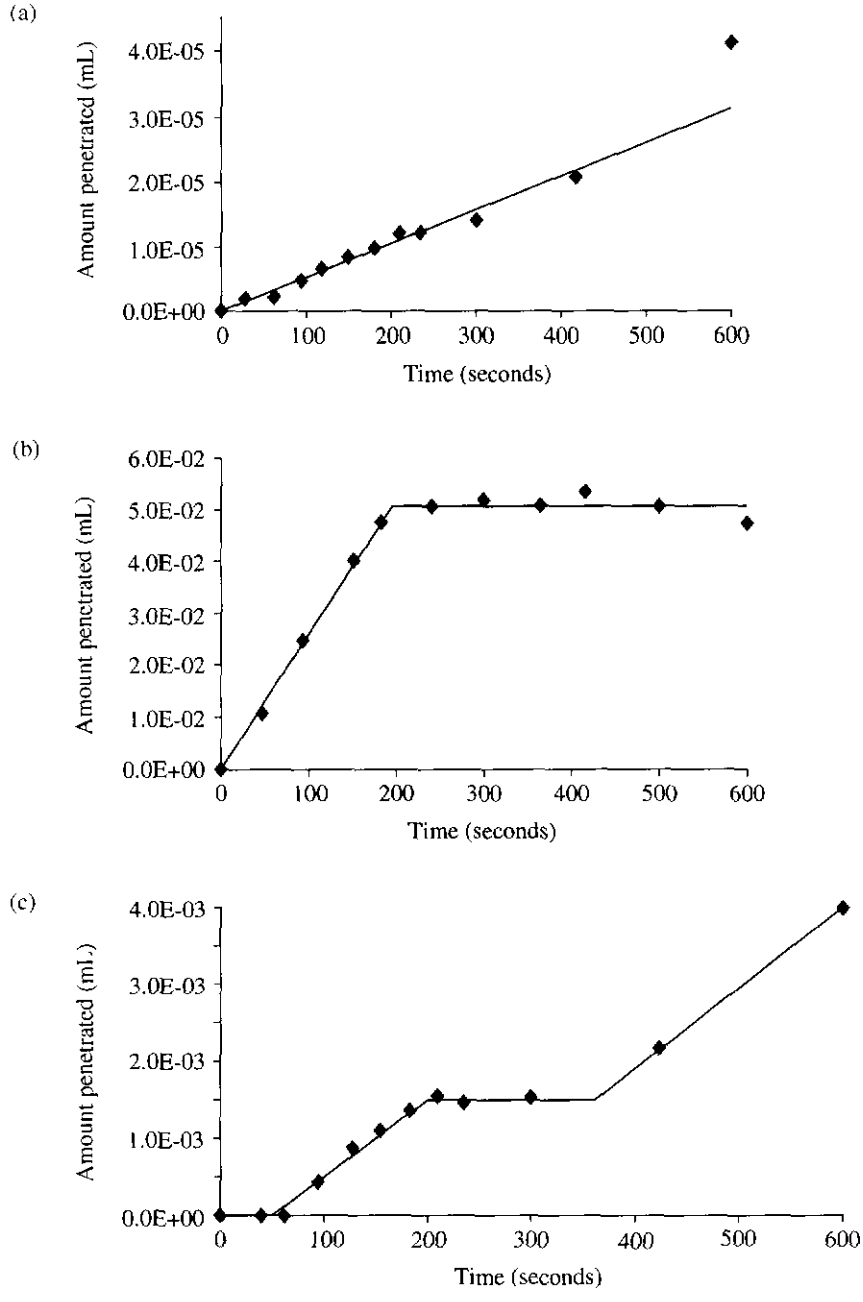

Figure 2. Three typical examples of viral passage kinetics obtained form tests performed at low pressure:
a) essentially linear viral penetration (2.6 micron hole);
b) linear initial penetration with complete cessation (28.1 micron hole), and
c) linear initial penetration, cessation, and resumption of penetration (15.6 micron hole).

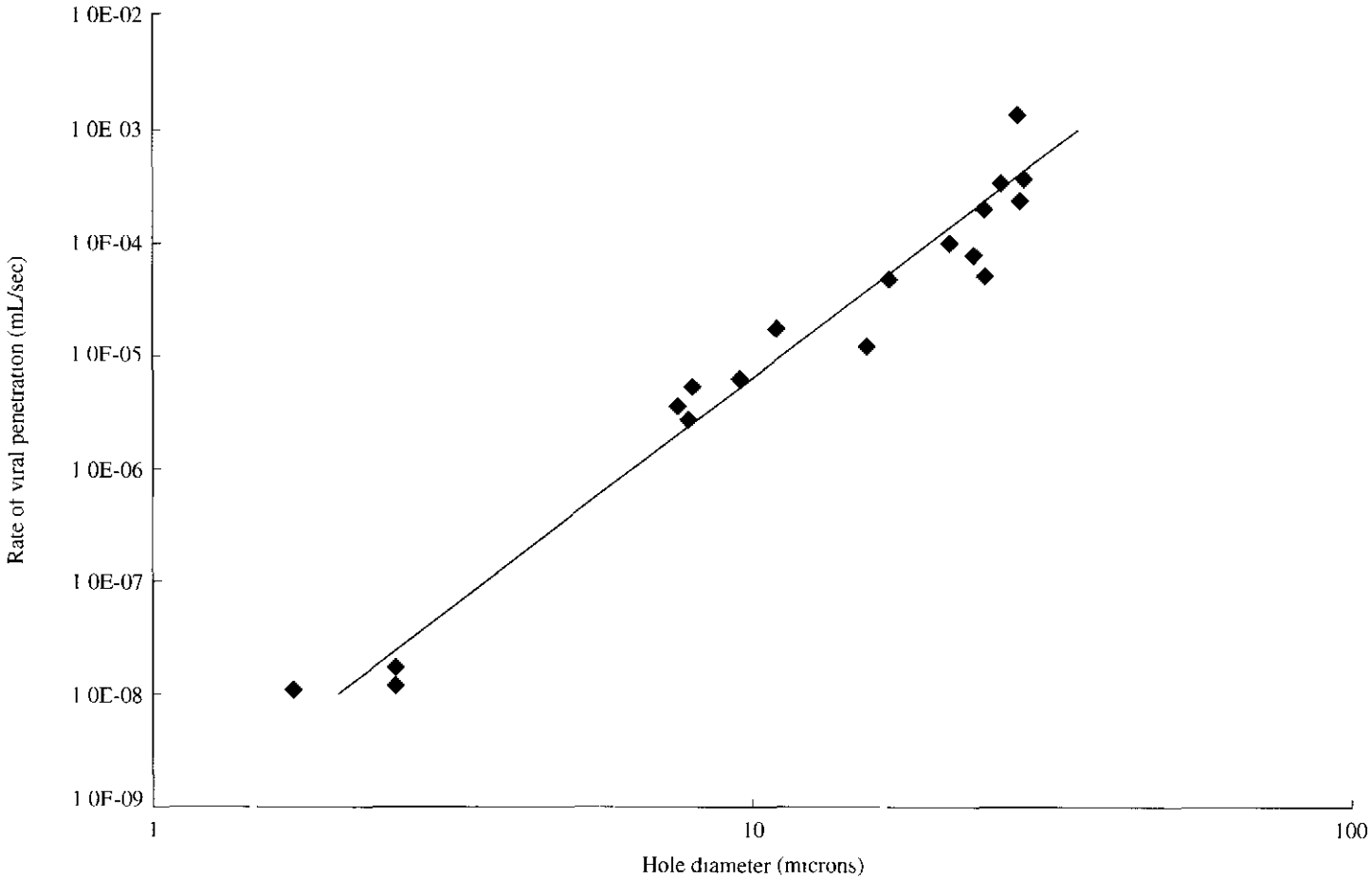

Figure 3 Rate of viral passage plotted as a function of hole diameter The rates of viral passage obtained from the low-pressure experiments were normalised to constant values of hydrostatic pressure (8.0 cm) and condom thickness i e hole length (95 microns) with the assumption that the passage rates were proportional to pressure and inversely proportional to hole length Hole diameters provided by Resonetics Inc

TABLE 1 MOLECULAR WEIGHT AND INTRINSIC VISCOSITY OF FRACTIONATED RUBBERS FROM FL-LATEX BEFORE AND AFTER TRANSESTERIFICATION

Rubber fraction	$\bar{M}_{w,LALLS} \times 10^{-5}$	$\bar{M}_{n,RI} \times 10^{-5}$	$\bar{M}_{n,osmo} \times 10^{-5}$	[η]
1	38.00	2.70	7.90	6 81
	(–)[a]	(–)	(–)	(–)
2	38 00	8.40	8.61	6 78
	(20.00)	(5 00)	(6 80)	(6.24)
3	40.00	6.60	5.50	6 55
	(29.00)	(4.30)	(3.90)	(6.22)
4	26.00	4.10	4.30	6.34
	(19.00)	(3 90)	(4.00)	(6.01)
5	24 00	3.70	3.40	6 11
	(19.00)	(1.80)	(2.10)	(5.77)
6	13.00	5.80	3.40	5.36
	(9.00)	(4.60)	(2.30)	(5.23)
7	8.40	2.70	2.70	4.19
	(–)	(–)	(–)	(–)
8	5 00	1.80	–	2.96
	(4.10)	(1.20)	(–)	(2 04)
9	3.30	1 70	1 20	2.45
	(2.70)	(1.40)	(1.02)	(1 33)
10	2.80	1.40	1 20	1.85
	(2.00)	(0.95)	(0.97)	(0.83)
11	1.60	1 00	–	1.19
	(–)	(–)	(–)	(–)
12	0 67	0 48	0.33	0.68
	(0.53)	(0.30)	(0.24)	(0.42)

[a]This sample fraction was not enough to analyse

Values in parentheses indicate intrinsic viscosities after transesterification

the whole molecule and that of each branch chain. The former is obtained by osmometry and the latter by ^{13}C-NMR from the ratio of *cis*- and *trans*-isoprene units, on the assumption of the presence of two *trans*-isoprene units at the initiating end of the rubber molecule[11]. Then, the number of branch-points (m) in the NR is determined from *Equation 3*:

$$m = \frac{M_n \text{ (osmometry)}}{M_n \text{ (^{13}C-NMR)}} - 1 \qquad \ldots 3$$

The relationship between m and the M_n determined by ^{13}C-NMR measurement was given as shown in *Figure 3* for deproteinised rubbers from FL- and HA-latices. It is clear that the m value of the rubber fractions from the deproteinised FL-latex was almost independent of the $\bar{M}_n$. On the other hand, it increased with increasing $\bar{M}_n$ value for the rubber fractions from deproteinised HA-latex. The maximum value of branch-points was estimated as much as five per rubber molecule

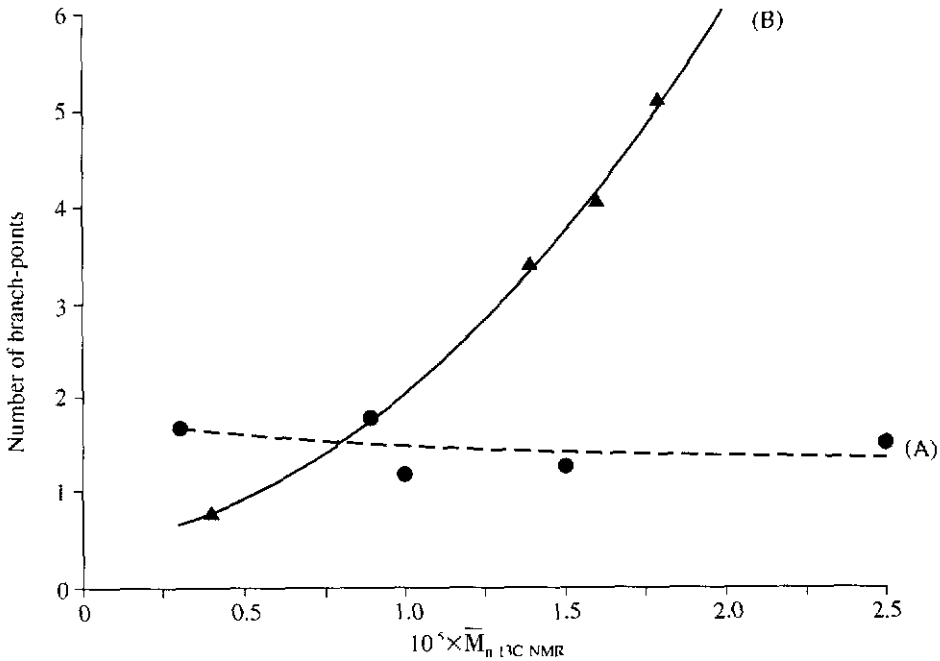

Figure 3. Relationship between the number of branch-points and $\overline{M}_n$ estimated by ^{13}C-NMR determined for the deproteinised rubber fractions from (A) FL-latices and (B) HA-latices.

for the highest molecular weight fraction. This difference implies that the number of branch-points increases during preservation of rubber latex in the presence of ammonia. This indicates that ammonia acts as an important function in the formation of branched molecules in NR latex.

We observed that the amount of fatty acid esters and phosphorus in deproteinised rubber, which is presumably present as a phospholipid, reduced to almost zero after transesterification[7]. The gel content of the transesterified rubber also decreased to about 1% and did not change even after a long period of storage. This result implies that all the branch-points are completely decomposed by treatment of the deproteinised rubber with sodium methoxide. This is strong evidence to convince us to conclude that phospholipid including fatty acids in NR is a predominant factor to originate branched-molecules. The structural analysis of these branch-points will reveal the mechanism of branching formation.

CONCLUSION

The branch-points were estimated to be two for deproteinised rubber from FL-latex and from two to five for that from HA-latex. All the branch-points are confirmed to be completely decomposed after deproteinisation followed by transesterification.

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