J. nat. Rubb. Res., **12(1)**, 11–20

Distribution and Origin of Abnormal Groups in Natural Rubber

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Ester and aldehyde groups are found to remain in natural rubber even after purification by deproteinisation and acetone extraction. Rubber isolated from smaller latex particles in the serum fraction contains a lower level of ester group than that from larger latex particles. Both aldehyde and ester groups are found to have a similar distribution in fractionated natural rubbers of different molecular weights. The concentration of these groups decreases with decreasing molecular weight of the rubbers. This finding suggests that the aldehyde groups are not derived from oxidative degradation of natural rubber. A drastic reduction in aldehyde content of natural rubber after transesterification is observed showing that the aldehyde groups are derived from oxidative degradation of olefinic group of unsaturated fatty acids bonded to the rubber molecule.

The main component of natural rubber molecule is *cis* polyisoprene hydrocarbon. Structurally, this natural polymer is more complicated than its synthetic analogue due to the presence of a small quantity of non-rubber groups, normally referred to as abnormal groups, bonded to the main-chain molecule. These groups are believed to be of biological significance in the biosynthesis of rubber.

It is now generally accepted that crosslinking reactions of the abnormal groups are the major cause for the formation of branching in natural rubber[1]. These branching entities eventually lead to the formation of gel and the occurrence of storage hardening of natural rubber which distinguishes it from the synthetic *cis* polyisoprene. The formation of gel during storage of dry rubber may involve a mechanism which is different from that of microgel in latex because the former is accelerated under low humidity conditions while the latter occurs in the aqueous medium[1].

Despite many years of investigation carried out by various workers, the mechanism for the gel formation has yet to be conclusively explained[1]. Nevertheless, several abnormal groups have been reported to be present in the main-chain rubber molecule and these are summarised in *Table 1*.

Since the presence of abnormal groups other than ester and aldehyde in natural rubber molecule is only circumstantial, the aim of this work is to provide more information on the distribution and the possible origin of these groups in natural rubber.

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TABLE 1. ABNORMAL GROUPS IN NATURAL RUBBER

Groups	Fractions	Concentration/ mmol kg^{-1}	Method	Ref.
Fatty acids	Low Mw	10–20	^{13}C-NMR	2
Lactone	Gel	10–15	IR	3
Aldehyde	Whole	10–35	H_2NOH	4
		1.5–5.0	2,4-DNPH	5
Amine	Whole	20–35	HBr titration	6
Epoxide	Whole	45–75	HBr titration	6
		10–15	Degradation	7

2,4-DNPH: 2,4-dinitrophenylhydrazine

MATERIALS AND METHODS

Field latex of RRIM 600 clone was preserved in 0.7% ammonia for 5 days before deproteinisation was carried out. Pale crepe rubber was of commercial grade and used as received. Other reagents were of analytical grade and used without further purification.

Synthesis of 2,4-dinitrophenylhydrazine-acetaldehyde (DNPH-acetaldehyde) Derivative[8]

Approximately 1.25 g of DNPH was dissolved in 25 ml of methanol containing 1 ml of sulphuric acid and filtered. It was then added drop-wise into 5 ml of vigorously stirred methanol containing 0.28 g of acetaldehyde. The precipitate formed was filtered and purified by recrystallisation from 95% ethanol. It was dried under reduced pressure at room temperature to constant weight. The melting point of the hydrazone was found to be 147°C which is comparable to the literature value[9] of 146°C–147°C.

Determination of Molar Extinction Coefficient of DNPH-acetaldehyde Derivative

The characteristics of the UV absorption of DNPH-acetaldehyde derivative in tetrahydrofuran (THF) was studied with a Jasco U-best 30 double beam UV-Vis spectrometer. A series of the derivative solutions with concentration up to 5.8×10^{-5} mol dm^{-3} was scanned from 600 nm to 200 nm. The plot of absorbance of the derivative against its concentration produces a molar extinction coefficient of 2.16×10^4 $dm^3mol^{-1}cm^{-1}$ and λ_{max} value of 359 nm, both of which are close to the literature values of $2.13 \times 10^4 dm^3mol^{-1}cm^{-1}$ and 360 nm, respectively, for butanal derivative[10].

Determination of Aldehyde Content of Natural Rubber

This method involves the treatment of 5 ml of 4% toluene rubber solution in a glass tube with

5 ml of 1% DNPH in THF. The details have been described elsewhere[5].

Fourier Transform Infrared (FT-IR) Analysis of Ester Groups in Natural Rubber

Methyl stearate was used as a model compound for the FT-IR analysis of ester groups in the natural rubber The absorbance of the carbonyl group of methyl stearate was measured in purified natural rubber obtained from serum fraction because this rubber was found to contain a very low level of ester groups as compared to that of cream and bottom fractions

The rubber samples for FT-IR analysis were prepared by casting 0.6% of the rubber solutions in chloroform on a KBr disk placed on activated silica gel and drying under a stream of nitrogen gas to form a round transparent film of about 1.5 cm in diameter The film was scanned with a Jasco 5300 FT-IR spectrometer at a resolution of 2 cm^{-1}. The spectrum obtained is the average of 300 scans.

The area ratio of peaks at 1738 cm^{-1} (C=O) to 1664 cm^{-1} (C=C), (A_{1738}/A_{1664}), was plotted against the concentration of the added ester groups in the rubber.

In the case of natural rubber containing unknown amount of ester groups, the FT-IR spectrum was obtained by the same procedure described above. Ester content was then obtained by substituting the area ratio of peaks at 1738 cm^{-1} to 1664 cm^{-1} into the following expression

$$\text{Ester (mmol/kg)} = (A_{1738}/A_{1664}) / \text{Gradient of calibration curve} \quad 1$$

Measurement of Gel Content

Rubber sample was allowed to dissolve in toluene at 0.3% w/v for one week in the dark The toluene solution was then centrifuged at 13 000 r.p m. (20 000 g) for 40 min. The sol and gel fractions were separated and the gel was dried under reduced pressure to constant weight The percentage of gel fraction was calculated from the weight ratio of the gel fraction to the original sample.

Isolation of Different Fractions of Latex

Fresh latex of about 10% dry rubber content (DRC) was poured into a condom placed in a centrifugal tube of similar size It was centrifuged at 13 000 r.p m. (20 000 g) for 30 min at room temperature Upon completion, it was immediately frozen in a freezer The condom was then removed from the frozen latex The cream, serum and bottom fractions were separated and precipitated into ethanol The rubbers were purified by reprecipitation from hexane into ethanol.

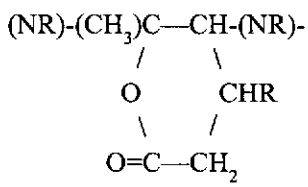
Other Measurements

Measurement of the molecular weight of rubber was carried out in toluene solution with a Wescan 231 Membrane Osmometer operating at 35°C using regenerated cellulose membrane The accuracy of the instrument was tested by the use of three standard polystyrene samples of known molecular weight. The results showed that the osmometer could perform within a deviation of 5% ^{13}C-NMR measurements were performed on deuterated chloroform solution of rubbers with tetramethylsilane (TMS) as an internal standard with a JEOL FX-200 NMR spectrometer operating at 50 1 MHz The sample concentration and pulse interval were 10% w/v and 12 seconds, respectively

RESULTS AND DISCUSSION

Ester Groups in Natural Rubber

The presence of ester groups in commercial natural rubber was first reported by Gregg and Macey[3]. However, they attributed the infrared band at 1738 cm^{-1} in the spectra of commercial rubber to the presence of lactone groups in the main-chain molecule as shown below:



where NR is the *cis* polyisoprene chain. However, ^{13}C-NMR studies on deproteinised natural rubber (DPNR) revealed that the ester groups in natural rubber are associated with the fatty acids which could be removed by transesterification with sodium methoxide[11]. On the other hand, if ester groups were of lactone origin, these groups would remain in the polymer chain even after treatment of the rubber with sodium methoxide.

Evidence for the presence of fatty acid ester groups in natural rubber is shown in *Figure 1* which shows ^{13}C-NMR spectra of whole fraction of DPNR and transesterified rubber obtained from fresh latex. Small signals at 14.0, 29.7 and 34.5 p.p.m. were assigned to terminal methyl ($-CH_3$), methylene ($-(CH2)_n-$) and methylene ($-O_2C\underline{C}H_2-$) carbons of long chain fatty acid, respectively. These signals disappeared after transesterification. Analysis of the methyl ester by ^1H-NMR revealed that it contains 20% unsaturated and 80% saturated fatty acids[11].

As shown in *Figure 2*, ester groups were found to remain in natural rubber even after extensive purification of latex with protease enzyme. *Alcalase 2.0T*, followed by successive washing with surfactant *via* centrifugation[12]. The DPNR showed a clear infrared band at 1738 cm^{-1}, which is a characteristic feature of carbonyl groups of fatty ester. Upon acetone extraction, about half of these groups remained in the polymer indicating that unbonded fatty acids were present in the purified rubber. The results on quantitative analyses of ester are shown in *Table 2*. The unextractable ester groups thus represent the level of bonded ester present in the rubber.

The ester content of natural rubber isolated from cream fraction, serum fraction and bottom fraction of centrifuged latex is indicated in *Table 3*. It is interesting to note that natural rubber from the serum fraction contained only 0.9 mmol/(kg rubber) of ester groups. This is much lower than those from cream and bottom fractions. Since the average diameter of rubber particles in the serum fraction has been shown to be about ten times smaller than that in the cream fraction[13], it is reasonable to assume that smaller particle size latex represents newly formed entity from which larger particle size latex is formed. Therefore, it is reasonable to predict that the esterification occurred after the rubber has been synthesised. This could account for the low ester content of rubber from smaller particle latex where only a small part of the rubber molecules had been esterified.

Distribution of Ester and Aldehyde Groups in Natural Rubber

The presence of aldehyde groups in natural rubber was proposed[4,5] because rubber-hydrazone was formed when the rubber was

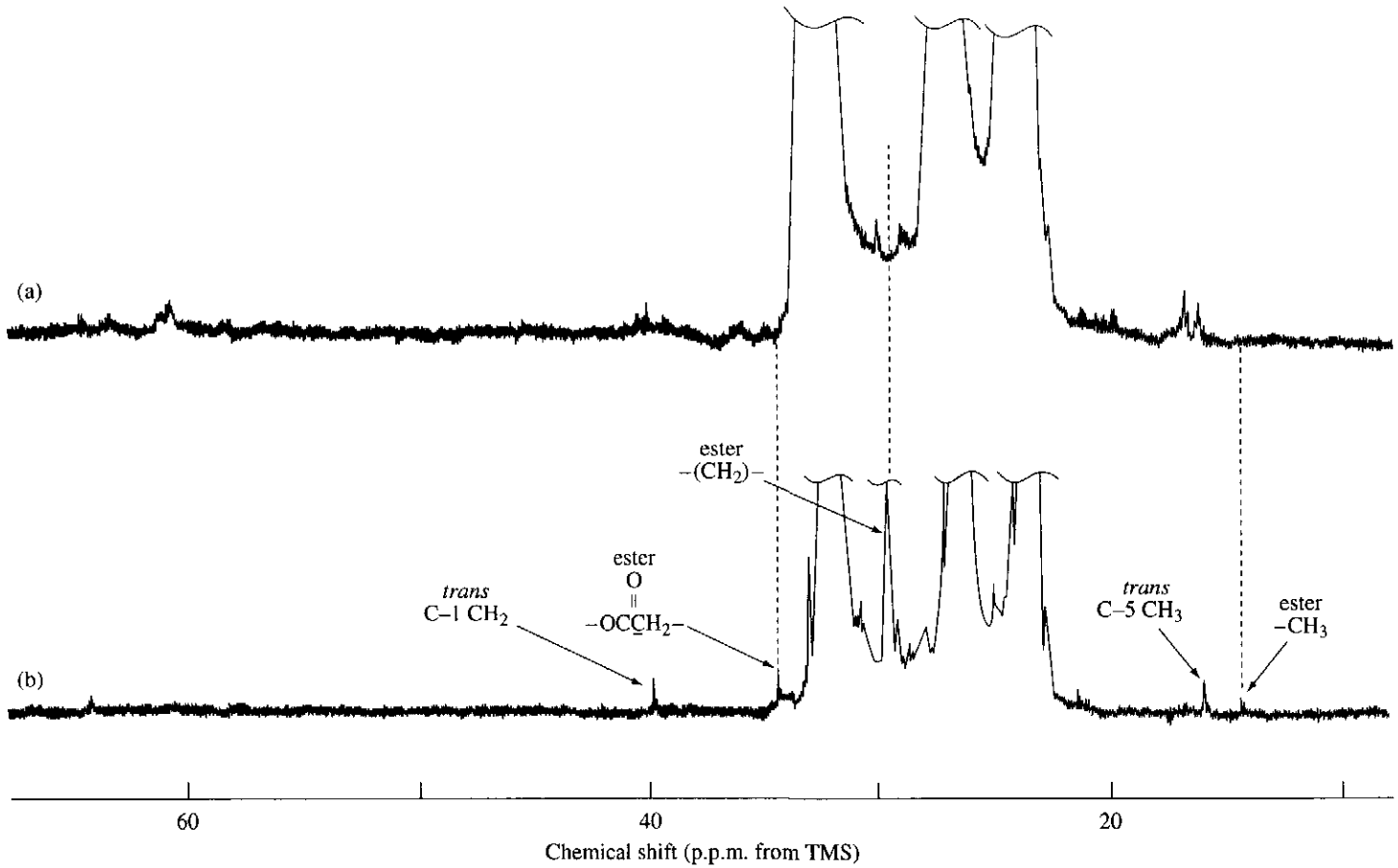


Figure 1 ^{13}C*-NMR spectra (a) transesterified natural rubber, and (b) deproteinised natural rubber.*

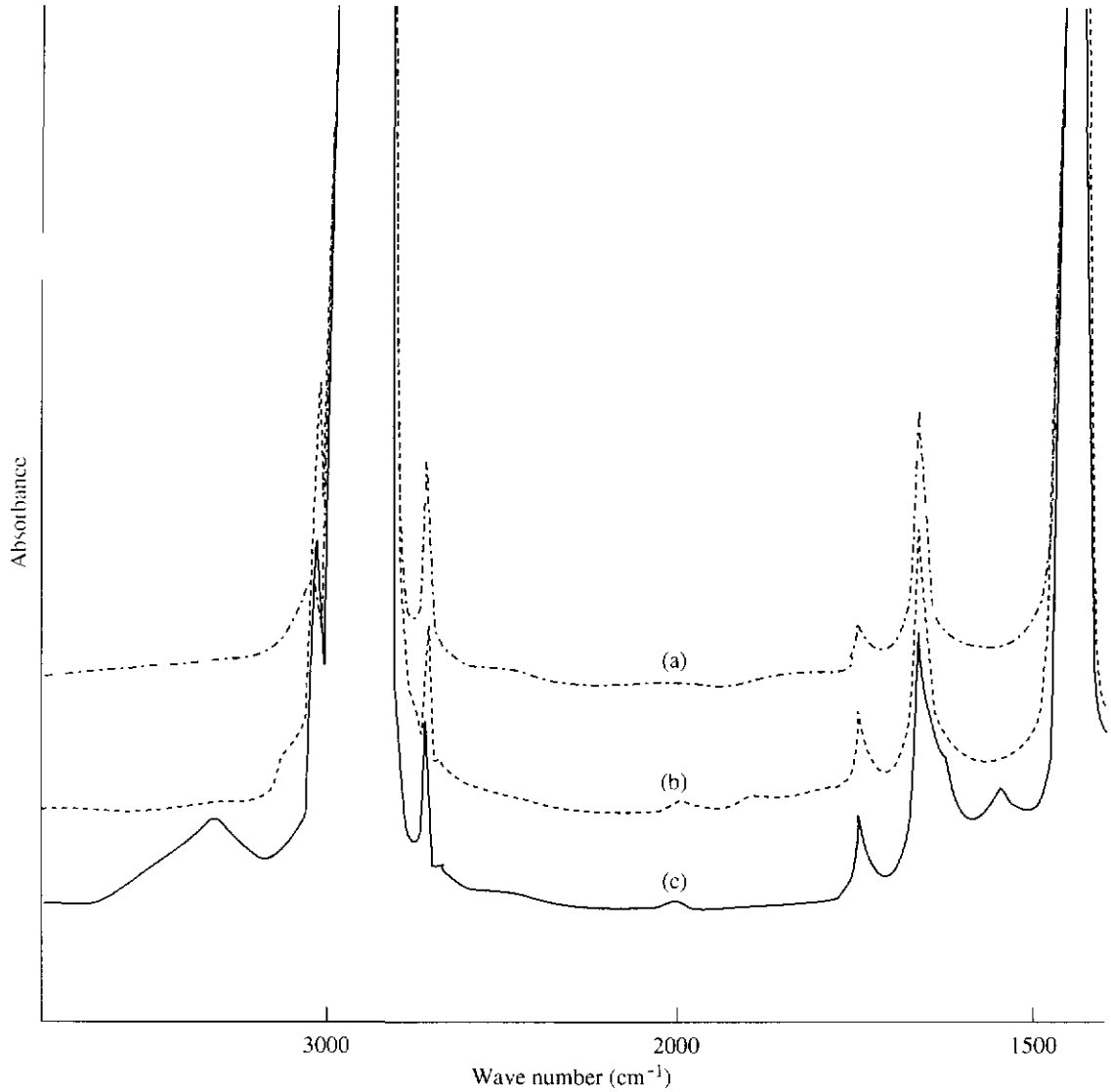


Figure 2. FT-IR Spectra (a) deproteinised and acetone extracted natural rubber (b) deproteinised natural rubber, and (c) control.

TABLE 2 ESTER CONTENT OF DIFFERENT NATURAL RUBBERS

Sample	Nitrogen content/% w/w	Ester content/mmol kg^{-1}
Control	0 30	18.0
DPNR	0 01	15 0
AE-DPNR	0 01	8 5

TABLE 3 ESTER CONTENT OF NATURAL RUBBER FROM CENTRIFUGED LATEX

Sample	Ester groups/ mmol $(kg\ NR)^{-1}$	Intrinsic viscosity
Cream fraction	7 8	7 1
Serum fraction	0.9	5 8
Bottom fraction	8.6	ND

ND Not determined

treated with 2,4-dinitrophenylhydrazine (DNPH) The estimated level of aldehyde was in the region of 1.6–5.4 mmol/(kg rubber) and the λ_{max} value of 353 nm–357 nm for the derivative suggests the presence of non-conjugated aldehyde.

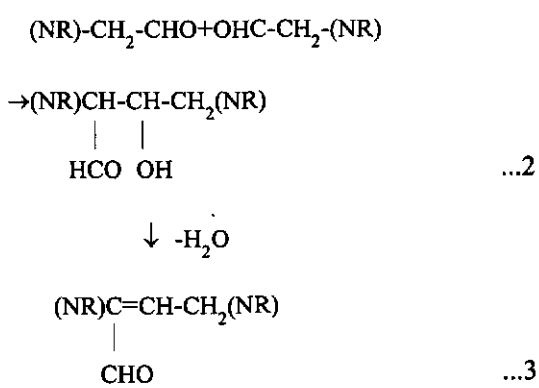
It has been demonstrated that the molecular weight of fractionated natural rubber before and after branching can be estimated by ^{13}C-NMR and membrane osmometry techniques, respectively[14]. These techniques were used in the present study to investigate the distribution of ester and aldehyde groups in fractionated natural rubbers. Thus, if two linear rubber molecules (molecular weight, *NR*) react to form a branched rubber molecule $[(NR)_2]$, the molecular weight of linear rubber molecule as analysed by ^{13}C-NMR will be *NR* and that analysed by osmometry will be $(NR)_2$.

Table 4 indicates that all fractions of the natural rubber contain an average of 1-2 ester groups and 0.2–0.4 aldehyde groups per linear rubber molecule based on two *trans* isoprene terminal units per chain[15]. The ester groups have been postulated to be located at the branching point of rubber molecule *via* association with phospholipid complex[14]. For branched rubber molecule, the aldehyde content increases with increasing molecular weight of the rubber indicating that the aldehyde groups are not auto-oxidative chain scission products of the natural rubber molecule because under these conditions, a reversed distribution order would be observed; *i.e.* low molecular weight rubber has high aldehyde content and *vice versa* Since high molecular weight rubbers have been found to contain more branching points than that of low molecular weight rubbers[14], the distribution of aldehyde groups

TABLE 4. ALDEHYDE AND ESTER GROUPS IN FRACTIONATED NATURAL RUBBER

Fraction	$M_n \times 10^{-5}$		Ester group/chain		Aldehyde group/chain	
	Linear	Branched	Linear	Branched	Linear	Branched
1	1.8	10.9	1.4	8.5	0.2	1.2
2	1.7	8.7	1.3	6.7	0.2	1.1
3	1.4	6.1	2.1	2.1	0.3	1.1
4	0.4	0.7	1.4	2.4	0.2	0.4

found here is in accordance with the postulation that branching entities of natural rubber are derived from aldo-condensation of the aldehyde groups[16]. However, under these circumstances, high molecular weight branched rubber molecule should contain more than one aldehyde group because the formation of each branching point produces one aldehyde group in the rubber molecule as demonstrated in *Equation 2*:



Furthermore, under the proposed reaction scheme, a conjugated aldehyde group is expected to be present as shown in *Equation 3*, but this was not observed in the hydrazone derivative obtained in the present study.

Since the trend of the distribution of the aldehyde groups is similar to that of ester groups in natural rubber, there is a possibility that these groups are derived from oxidation of olefinic groups of unsaturated fatty acids bonded to natural rubber molecule. The oxidation of bonded unsaturated fatty acids would not cause a significant decrease in the molecular weight of the rubber. This process may proceed *via* an enzyme assisted mechanism. The presence of oxidising enzymes, *i.e.* oxidases in natural rubber latex has been reported[17]. Therefore, if the high molecular weight fractions represent old rubber molecules in the tree, it is not surprising to find that more olefinic groups of the bonded unsaturated fatty acids have been oxidised in the presence of the oxidases to form aldehyde groups as compared to those in low molecular weight fractions.

If the aldehyde groups originated from oxidation of bonded unsaturated fatty acids, the removal of the rubber ester groups will drastically reduce the aldehyde content of the rubber. *Table 5* shows the aldehyde content of commercial pale crepe and DPNR from high ammonia preserved field latex before and after transesterification[14]. These results indicate that substantial amount of the aldehyde groups could be removed *via* transesterification. The presence of higher residual aldehyde groups in the pale crepe rubber than those in DPNR after

TABLE 5. ALDEHYDE CONTENT OF TRANSESTERIFIED NATURAL RUBBERS

Sample	Aldehyde content/mmol kg^{-1}
Pale crepe	4.6
TE Pale crepe	2.2
DPNR	2.4
TE-DPNR	0.4
Synthetic polyisoprene	0.3

TE: transesterified

transesterification is probably due to oxidative degradation of the pale crepe rubber during the production and storage of the rubber. In the case of DPNR, the level of aldehyde groups after transesterification is much lower, indicating that the aldehyde groups are associated with the ester groups bonded to natural rubber molecule.

CONCLUSION

Ester groups are found to remain in natural rubber after deproteinisation and acetone extraction. Rubber isolated from smaller latex particles in the serum fraction contains a lower level of ester groups than that from larger latex particles in the cream fraction. Both aldehyde and ester groups are found to have a similar distribution in fractionated natural rubbers of different molecular weights. The concentration of these groups decreases with decreasing molecular weight. It is concluded that the aldehyde groups are not derived from oxidative degradation of natural rubber, but are derived from oxidative degradation of olefinic groups of unsaturated fatty acids bonded to natural rubber.

Date of receipt: January 1997
Date of acceptance: August 1997

REFERENCES

1. BURFIELD, D.R. (1986) Storage Hardening of Natural Rubber: An Examination of Current Mechanistic Proposals. *J. nat. Rubb. Res* , **1(3)**, 202.

2. TANAKA, Y. (1984) Structural Characterisation of Naturally Occurring *Cis*-polyisoprenes. *ACS Symposium Series No. 247 'NMR and Macromolecules (Randall, J.C. ed.)*, p. 233. Washington: American Chemical Society.

3. GREGG, E.C JR. AND MACEY, J.H. (1973) The Relationship of Properties of Synthetic Polyisoprene and Natural Rubber in the Factory. The Effects of Non-rubber Constituents of Natural Rubber. *Rubb. Chem Technol.*, **46**, 47.

4. SEKHAR, B.C. (1960) Degradation and Crosslinking of Polyisoprene in *Hevea brasiliensis* Latex During Processing and Storage. *J. Polym. Sci.*, **48**, 133.

5. SUBRAMANIUM, A. (1977) Estimation of Aldehyde Groups in Natural Rubber with 2,4-dinitrophenylhydrazine. *J. Rubb. Res. Inst Malaysia*, **25(2)**, 61.

6. BURFIELD, D.R. AND GAN, S.N. (1977) Determination of Epoxy Groups in Natural Rubber by Degradation Methods. *Polymer*, **18**, 607.

7 BURFIELD, D R AND GAN, S N (1977) Non-oxidative Crosslinking Reactions in Natural Rubber II Radiotracer Study of the Reactions of Amino Acids in Rubber Latex *J Polym Sci Polym Chem Ed*, **15**, 2721

8 BURFIELD, D R AND LAW, K S (1979) Determination of Carbonyl Groups in Polymers by Reaction with 2,4-Dinitrophenyl Hydrazine *Polymer*, **20**, 621

9 JONES, L A AND HANCOCK, C K (1960) Spectrophotometric Studies of Some 2,4-Dinitrophenylhydrazones III. Taft and Hammett Relationships *J Org Chem*, **25**, 226.

10 ARNOLD, A R AND STAPLES, R (1992) Determination of Carbonyl as Their 2,4-Dinitrophenylhydrazine (DNPH) Derivatives in Peroxide Containing Polymers. *Polymer*, **33**, 1739.

11 TANAKA, Y, ENG, A H AND EJIRI, S (1992) Structural Characterisation of Natural Rubber *Proc Rubbercon 92, Brighton, U K*, 147

12 ENG, A H, TANAKA, Y AND GAN, S N (1992) FT-IR Studies on Amino Groups in Purified Natural Rubber *J nat Rubb Res*, **7**, 152

13 TANAKA, Y, KAWASAKI, A, HIOKI, Y, KANAMARU, E AND SHIBATA, K (1995) Highly Purified Natural Rubber II Effect of Small Rubber Particles *Proc Int Rubb Conf Kobe, Japan*, 247

14 ENG, A H, EJIRI, S, KAWAHARA, S AND TANAKA, Y (1994) Structural Characteristics of Natural Rubber Role of Ester Groups *J Appl Polym Sci Polym Symp*, **53**, 5

15. ENG, A H, KAWAHARA, S AND TANAKA Y (1994) *Trans* Isoprene Units in Natural Rubber *Rubb Chem Technol*, **67**, 159

16. GORTON, A D T (1974) The Viscosity of Rubber in Natural Rubber Latex Concentrate and its Modification II The Influence of Carbonyl Reagents and Peptisers *Rubb Ind*, 142

17 D'AUZAC, J AND JACOB, J L (1989) *Physiology of Rubber Tree Latex The Laticiferous Cell and Latex — A Model of Cytoplasm (d'Auzac, J, Jacob, J L and Chrestin, H eds)*, p 59 Florida CRC Press.