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Microstructure of Peroxide Prevulcanised Latex Films

P.E.F. CUDBY* AND R.T. DAVIES*#

The technique of styrene swelling/polymerisation/electron microscopy has been used to study the structure of films made from peroxide prevulcanised latex (PPVL). The results show that in PPVL which has been made by the usual method, i.e. *by using an activator in the aqueous phase, the distribution of crosslinked rubber is very different from that in sulphur-cured or radiation-cured prevulcanisates. In PPVL it can be seen that the small rubber particles are vulcanised in their entirety but the larger particles show a very different structure. Each large particle is only vulcanised in a layer near its surface, and the centre of the particle is unvulcanised or poorly vulcanised. This observation has been confirmed by solvent-swelling measurements on films made from different particle size fractions of PPVL. This phenomenon could explain why PPVL films show a high degree of tension set. By contrast, in a PPVL made using a more rubber-soluble, thermally-activated peroxide (dicumyl peroxide) all the rubber particles were crosslinked throughout.*

A method of visualising the vulcanised areas in unsaturated elastomers[1] has been used to estimate the crosslink density of vulcanised elastomer blends[2,3] and, more recently, to study the structure of various prevulcanised and post-vulcanised natural rubber (NR) latex films[4]. The procedure involves equilibrium swelling of the dried latex film with styrene followed by polymerisation of the styrene. This causes a phase separation between the rubber and the polystyrene which will be discussed in more detail later. The polymérisation also makes the sample rigid enough for an ultra-thin section to be cut at ambient temperature. The cut section is then stained with osmium tetroxide to show up the rubber network when observed by transmission electron microscopy (TEM). The first micrographs of PPVL films obtained by this method showed an unusual structure[4]. This paper describes subsequent work done on several PPVL films to provide a better understanding of the crosslink distribution in these materials.

MATERIALS AND METHODS

Most of the PPVLs were prepared by the method described previously[5,6]. The t-butyl hydroperoxide (tBHP) was added as a 70% aqueous solution *(Interox)* to 20% potassium laurate solution and half the water required to dilute the latex to 50% solids content. The amount of potassium laurate used was 0.25 parts per hundred rubber (p.p.h.r.). This mixture

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was added with stirring to a commercial HA (High ammonia) latex concentrate at 60°C. The fructose was added as a 20% solution at the start of the reaction followed by the rest of the water. A fructose/tBHP mole ratio of 0.6 was used unless otherwise stated. The mixture was stirred continuously (under a sheet of polythene to reduce evaporation) for 7 h and a few drops of concentrated ammonia solution were added occasionally to compensate for evaporation losses. The PPVL used to make the films in *Figures 1* and *2* was made by using 7mmol tBHP/100 g rubber which was all added to the latex at the start of the reaction. The PPVL film in *Figure 3* was made using 5mmol tBHP/ 100 g rubber which was added to the latex in five equal hourly aliquots during the first 4 h of the reaction. Cast, unleached films of both PPVLs were used for the electron microscopy analysis.

The cumene hydroperoxide (CHP) PPVL was made in a similar way but in this case it was necessary to emulsify the 80% CHP solution *(Aldrich)* with the soap (potassium caprylate at 0.25 p.p.h.r.) and half of the water before adding dropwise to the HA latex at room temperature with stirring. After stirring for 45 min, the temperature was raised to 60°C and stirring was continued for another 45 min. Then the fructose and the rest of the water were added and the mixture was stirred at 60°C for 7 h. A CHP concentration of 10mmol/100 g rubber and a fructose/CHP mole ration of 1.0 were used.

The highly crosslinked PPVL was made by adding a mixture of the tBHP, potassium laurate and water in five equal aliquots (one every 45 min) to the HA latex in a screw-cap bottle kept in a water-bath at 60°C. All the fructose was added at the start. After each addition of ingredients the bottle was flushed with nitrogen, capped and then swirled to mix. The heat was switched off after 8 h. A tBHP concentration of 20mmol/100 g rubber and a fructose/tBHP mole ratio of 0.6 was used.

Latex was prevulcanised with dicumyl peroxide (DICUP) as follows. The HA latex was diluted to 50% total solids content with water and then a 50% solution of DICUP in toluene was added dropwise with stirring at room temperature in a screw-cap bottle. The bottle was flushed with nitrogen, capped then rotated on rollers for six days at ambient temperature. After flushing with nitrogen again the mixture was prevulcanised by heating in an oven at 70°C for nine days. A DICUP concentration of 12mmol/100 g rubber was used.

Sulphur-prevulcanised latices of medium modulus (MR *Revultex*) and low modulus (LR *Revultex*) were supplied by Revertex (Malaysia) Sdn Bhd. RVNRL was provided by the Japan Atomic Energy Institute.

The film of LR Revultex was made by dipping a glass plate into a solution of calcium nitrate in ethanol, drying for 30 sec at 70°C, then dipping into the latex to form a coagulated film. The film was leached as a wet gel for 15 min at room temperature and then dried at 70°C.

Cast films were prepared by pouring latex at 50% total solids content onto a level glass plate and drying at room temperature. Unless otherwise stated, films were leached by soaking in distilled water for two 24-hour periods at room temperature followed by a 30-minute soak in fresh distilled water at 50°C. Films were dried at 70°C.

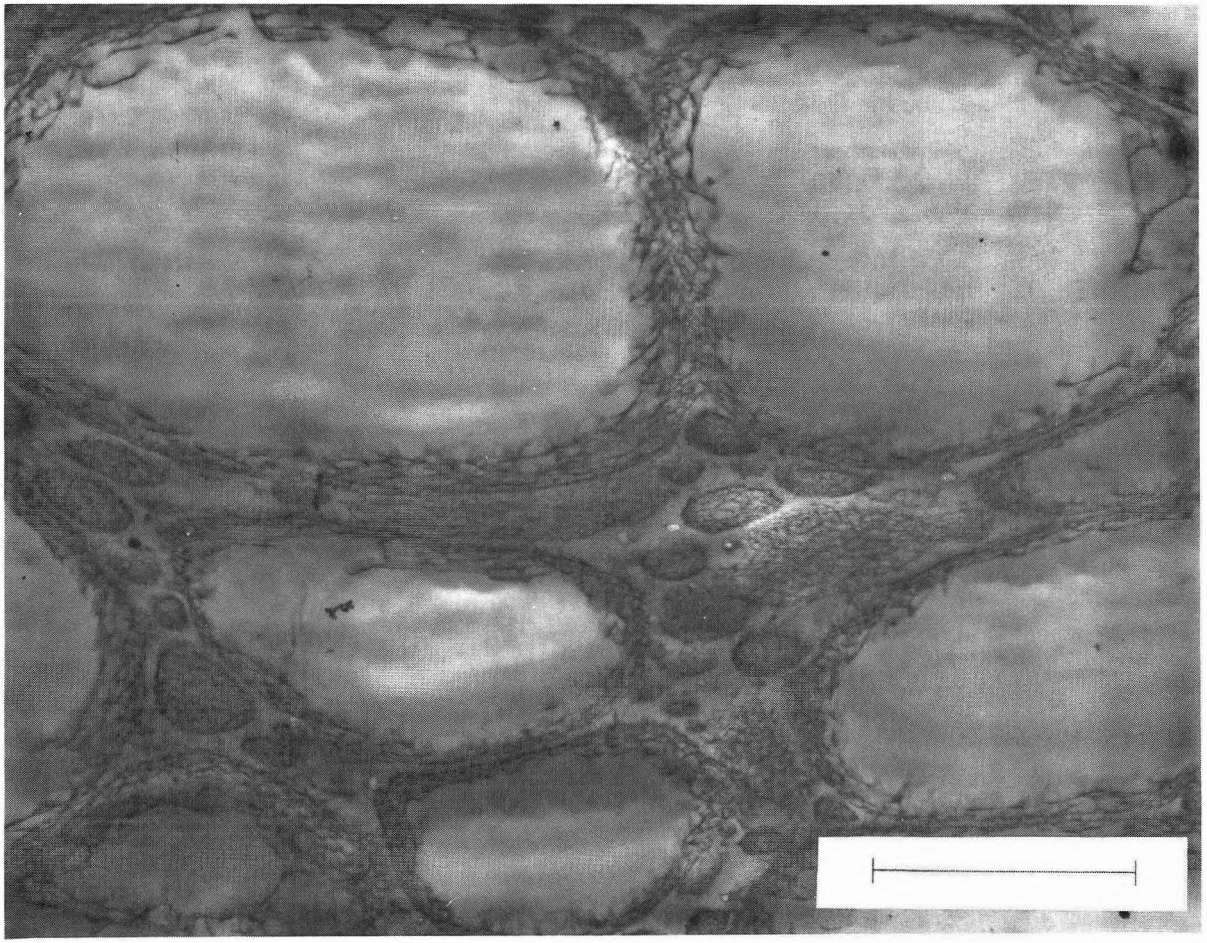

Figure 1. Cast, unleached PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

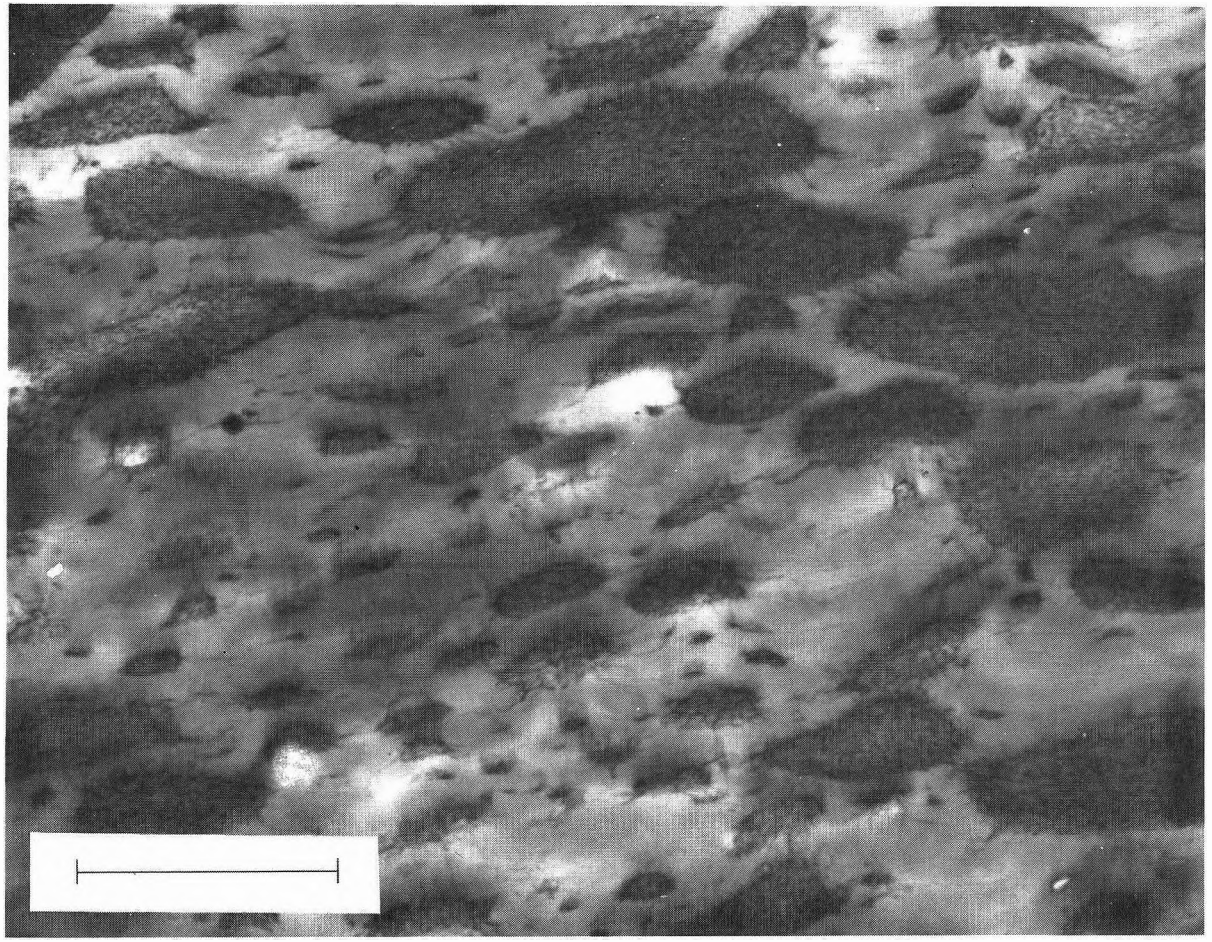

Figure 2. Dipped and leached LR Revultex *film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.*

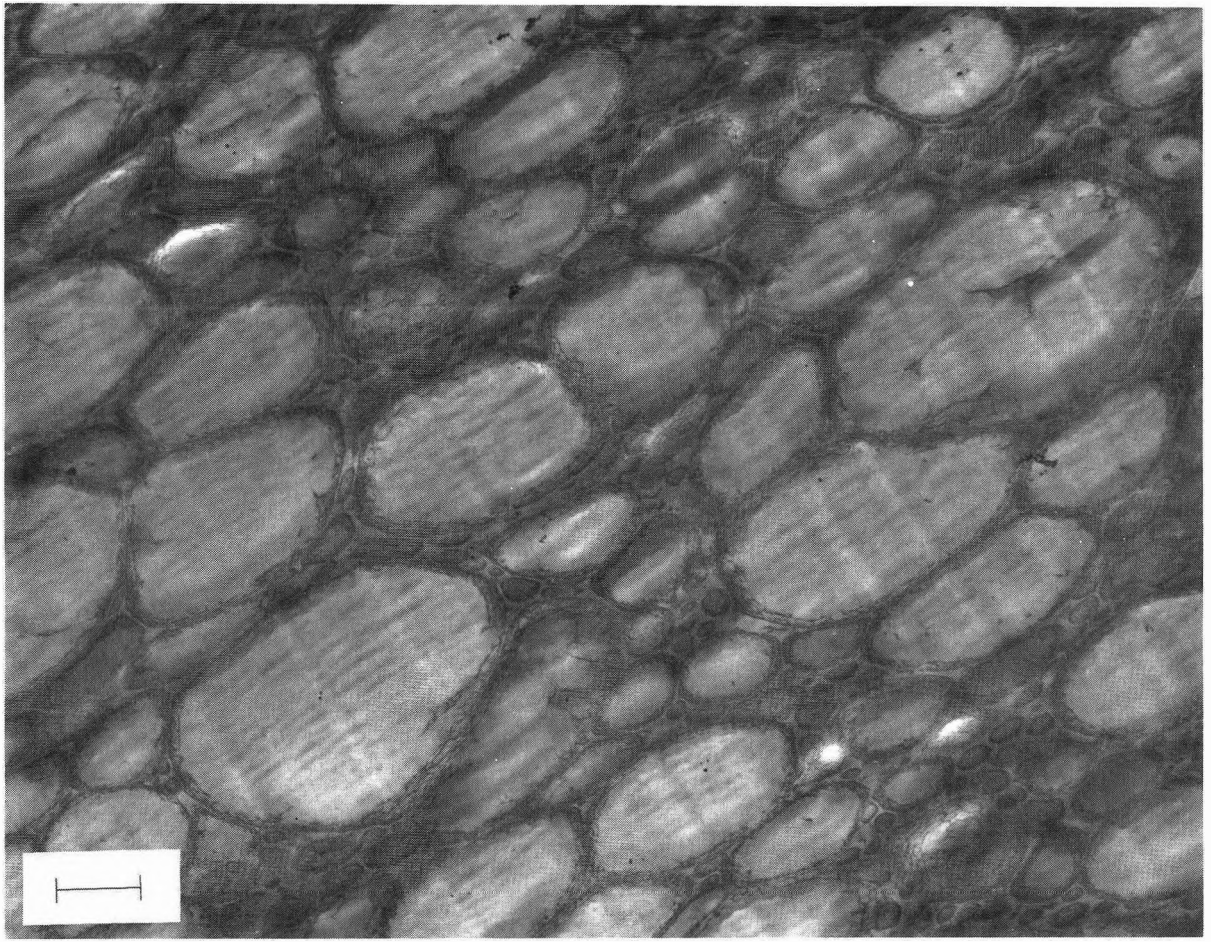

Figure 3. Cast unleached PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

Samples of dried latex film were prepared for styrene swelling and electron microscopy as follows. A small piece of rubber was swollen to equilibrium in a 1% w/w solution of benzoyl peroxide in styrene containing 10 p.p.m. – 15 p.p.m. of 4-tert-butylcatechol inhibitor as supplied (99%, *Aldrich*) and 2% w/w of the plasticiser di-n-octyl phthalate. The plasticiser was added to make the polystyrene less brittle and therefore easier to section. Portions of the swollen rubber in excess styrene were heated in gelatine capsules at 68°C for 16 h. Each sample was heated for an additional 4 h at 90°C to complete the polymerisation. Ultra-thin sections (about 100 nm – 150 nm thick) were cut from the swollen, embedded sample at ambient temperature with an RMC MT-7000 ultramicrotome using a 45° freshly cleaved glass knife set at a shallow clearance angle. Where possible, sections were carefully relaxed, while still in the knife trough, by brief exposure to low levels of xylene vapour from a wick which had been dipped in xylene. The wick was removed immediately after any visible relaxation occurred. The sections were collected on nickel grids and stained with osmium tetroxide vapour for 2 h prior to examination in a Phillips EM300 transmission electron microscope operating at 100 kV.

Samples of latex film (not swollen) were prepared for uranyl acetate staining and electron microscopy as follows. Ultra-thin sections were cut cryogenically with an RMC MT-7000 microtome fitted with an RMC CR-21 cryo-kit. Freshly cleaved 45° glass knives were used at a shallow clearance angle. Sections were cut dry (*i.e.* with no trough or trough liquid) and mounted on nickel grids. These were placed in a freshly made, saturated uranyl acetate solution (70% ethanol/30% water) for 2 h and then washed in 70% ethanol/30% water prior to examination in a Phillips EM300 transmission electron microscope operating at 100 kV.

Centrifugal fractionation of latex was carried out by diluting to 30% total solids content (TSC) with 1.8% aqueous ammonia, and transferring to 35 ml centrifuge tubes. These were centrifuged at 4000 r.p.m. for 10 min on a BTL bench centrifuge. The large particle size fraction was prepared as follows; the cream layer was removed with a spatula, redispersed in 1.8% ammonia and the centrifugation was repeated. The cream was removed and coagulated with dilute acetic acid. The coagulum was pressed flat and leached for 2 x 24 h in distilled water at room temperature followed by half an hour in distilled water at 50°C before drying in air at 25°C. The small particle size fraction was prepared as follows. The top 3 cm of latex was discarded after the first centrifugation and removal of the cream. The remaining latex was separated from the visible sediment, diluted with 1.8% ammonia and transferred to ultracentrifuge tubes. The latex was then centrifuged at 10 000 r.p.m. for 10 min in a Beckman L2-65B ultracentrifuge. The resulting latex fraction was decanted, leaving a top layer of coagulated rubber and a bottom layer of sediment behind. This latex fraction was diluted with 1.8% ammonia and centrifuged at 35 000 r.p.m. for 30 min to ensure that it was free of sediment. The creamed latex was then scooped out of the top of the tubes, coagulated with dilute acetic acid and then pressed, leached and dried in the same way as the top fraction.

The Q values of each film were measured by immersing a small (*ca.* 0.2 g) piece of dry latex film in excess toluene (BDH Analar grade) for 16 h at 25°C ± 2°C and weighing

the amount of toluene absorbed. The Q value is defined as the ratio of the weight of toluene absorbed to the weight of the dry film.

RESULTS AND DISCUSSION

A typical electron micrograph of a polymerised styrene-swollen film of PPVL which had been made by using an activated tBHP system is shown in *Figure 1*. The dark areas in the micrograph correspond to rubber which has been stained with osmium tetroxide and the light areas are mainly polystyrene. The ovoid shape of the particles is probably a result of the sectioning process wherein the action of the knife edge tends to slightly compress the sample. The equilibrium volume swelling ratio Q of the film used to obtain this micrograph was 5.8, similar to that of a typical low-modulus sulphur-prevulcanised latex film such as LR *Revultex*. However, the electron micrographs of these two film after styrene swelling and polymerisation (*Figures 1* and *2*) show a great difference in structure. Whereas in sulphur-prevulcanised latex films a reasonably uniform network of rubber is seen in each latex particle, in PPVL only the smaller particles have this uniform network structure, while the large particles show a very different, inhomogeneous structure.

The structure seen in the sulphur prevulcanisates is believed to arise from the following processes. During the styrene-swelling stage the rubber and the styrene are fully miscible so styrene swells the vulcanised latex particles until the entropic driving force is balanced by the elastic restoring force of the rubber network. During polymerisation a polystyrene phase is created which is immiscible with the rubber. Thus a phase separation occurs in which rubber chains are forced together between the growing domains of polystyrene, resulting in a mesh structure of rubber threads that is visible under the electron microscope. Threads of rubber are clearly present in between the latex particles as well, but here there is a much greater amount of polystyrene. This is believed to arise partly from styrene-swelling of the thin layer of entangled rubber chains between the latex particles, and partly from migration of polystyrene out of the latex particles during the polymerisation stage.

In sulphur and radiation prevulcanisates all the latex particles show a homogeneous network structure[4] but in the PPVL sample only the small latex particles have this appearance. The rubber network in the larger latex particles is concentrated near the particle boundaries.

It is instructive to compare a picture of the same styrene-swollen PPVL film at a lower magnification *(Figure 3)* with one of an unswollen PPVL film of similar Q value at the same lower magnification *(Figure 4)*. The latter was sectioned and then treated with uranyl acetate, a compound which stains the protein in the film, thus delineating the particle boundaries. *Figure 4* can therefore be used to give an idea of the size range of the rubber particles in the film, though the apparent size of each particle will depend on the level at which the particle was sliced during sectioning. It is clear that the largest particles in *Figure 3* are bigger than those in *Figure 4*. This is in contrast to the situation in sulphur-cured or radiation-cured prevulcanisates where the rubber particles themselves do not show a great increase in size and the bulk of the polystyrene occupies the spaces between the particles[4].

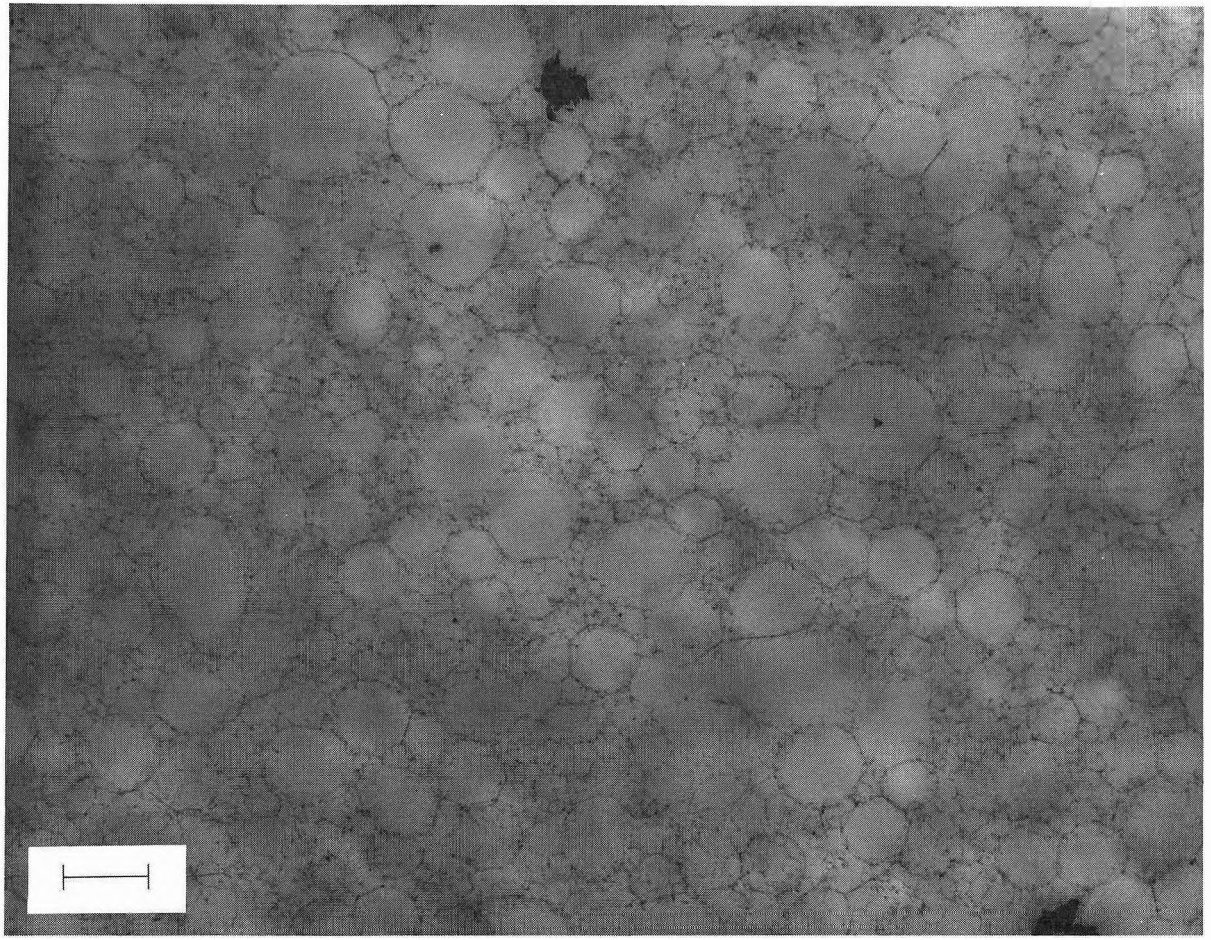

Figure 4. Cast, unleached PPVL film stained with uranyl acetate. Scale bar is 1 micron.

The distinctive PPVL structure can be explained if the large rubber particles in PPVL have unvulcanised, or poorly vulcanised, centres. Thus the following scenario is proposed for the changes that take place in a PPVL film during the sample preparation process. During the swelling of the latex film by styrene, the central regions of the larger particles become swollen to a greater extent than the more fully vulcanised small particles and outer regions of larger particles. During polymerisation of the styrene the phase separation produces separate domains of rubber and polystyrene. The small uniformly vulcanised latex particles behave in the same way as the particles in a sulphur prevulcanisate, as described above. However each large particle contains a central mass of rubber chains dissolved in a large amount of styrene. As polystyrene forms inside the particle this rubber can be easily displaced to join the network of vulcanised rubber near the particle surface, leaving behind a large solid mass of polystyrene at the centre of the particle.

If the large rubber particles in PPVL really have unvulcanised, or poorly vulcanised cores as the micrographs (*Figures 1* and *3*) suggest, then a latex film made from large particles should have a higher solvent swelling index, Q, than a film made from smaller particles. To test this, a sample of PPVL and a sample of sulphur prevulcanised latex (MR *Revultex*) were fractionated by centrifugation. The Q values of films made from the large and small particle size fractions are given in *Table 1*.

The results show that whilst the swelling ratio of sulphur-prevulcanised latex films is independent of latex particle size, the large particle size fraction of PPVL has a much higher swelling ratio than the small particle size fraction from the same latex. These results are consistent with the hypothesis that the centres of large rubber particles do not become substantially vulcanised during the normal peroxide prevulcanisation process.

The inhomogeneous nature of the crosslinking in PPVL is not entirely surprising. The tBHP, fructose and trace amounts of iron are all dissolved in the aqueous serum of the latex. Formation of the alkoxy radicals which initiate the crosslinking process therefore occurs in the aqueous phase. These alkoxy radicals react first with the rubber molecules on the surface of the latex particles, removing hydrogen atoms to produce rubber radicals which combine to form crosslinks. The electron micrographs suggest that crosslinking occurs before the alkoxy radicals or rubber radicals can diffuse very far into the rubber particles. In the case of the small particles it seems that the distance that the radicals can diffuse is large enough to result in crosslinking throughout each particle.

In the light of the results presented herein and the above explanation, it is perhaps surprising that the physical properties of PPVL films are as good as they are[5,6]. However, it has not been possible to make a PPVL film which combines high tensile strength and high modulus. Also PPVL films have been found to possess a relatively high degree of tension set which can probably be accounted for by the domains of unvulcanised or poorly vulcanised rubber. For example, it has been noted that toy balloons made from PPVL have similar properties to balloons made from sulphur prevulcanised latex except that after deflation the PPVL balloons are larger than sulphur-prevulcanised ones of similar strength and thickness. Whilst this may not be a problem

TABLE 1. SOLVENT-SWELLING INDICES OF LATEX FILMS

Item	PPVL	Sulphur prevulcanisate
Whole Latex	5.9	5.2
Large rubber particle fraction	7.1	5.4
Small rubber particle fraction	3.2	5.3

for balloons, and may even be an advantage in that it would permit faster environmental degradation of burst balloons, high tension set would not be acceptable in other latex products. Some attempts were therefore made to increase the depth of the vulcanised layer in PPVL.

First, the effect of increasing the degree of vulcanisation (as measured by the swelling index) was tested. By using higher concentrations of tBHP and fructose, a highly-crosslinked PPVL with a Q value of 3.3 was made. Micrographs derived from cast, unleached and water-leached films made from this PPVL (*Figures 5* and *6*) show that the vulcanised layer has not extended noticeably further into the particles. The finer mesh size within the small particles in this film compared to *Figure 1* confirms that a higher crosslink density has been achieved[4]. In the highly crosslinked PPVL there is an interesting difference between unleached and leached films. In the unleached film *(Figure 5)* more polystyrene is visible in the interstices between the rubber particles than in the leached film *(Figure 6)*. In the latter it appears that the small, fully vulcanised particles have merged to form an almost homogeneous mass of crosslinked rubber between the larger, styrene-swollen particles. It is possible that the leaching process, by removing some of the hydrophilic substances from the surfaces of the particles, has increased the amount of rubber-to-rubber contact between the particles. This would reduce the tendency of the polystyrene to migrate from the interior of the particles to the areas between them during polymerisation.

In another attempt to increase the depth of the vulcanised layer, the more rubber-soluble cumene hydroperoxide was used in place of tBHP for the prevulcanisation. The resulting film, which had a Q value of 6.0, gave a micrograph *(Figure 7)* showing the same pattern as had been seen for the other PPVL films. This result suggests that it is not the solubility of the peroxide in the rubber which is the significant factor, but the fact that the activation process takes place in the aqueous phase. In order to confirm this, a PPVL was produced using a rubber soluble peroxide, DICUP, with heat rather than an aqueous redox reaction to provide the initiation. Unfortunately, thermal scission of DICUP is slow and inefficient at 70°C. Nevertheless, it was possible to produce a lightly prevulcanised latex which yielded a cast film with a solvent-swelling ratio of 11.0. The electron micrograph of this film after styrene swelling and polymerisation *(Figure 8)* shows a reasonably homogeneous network within each particle and large areas of polystyrene between the particles. The pattern in this micrograph is similar to those obtained from sulphur and radiation vulcanised latex[4], but different from other micrographs derived from PPVL.

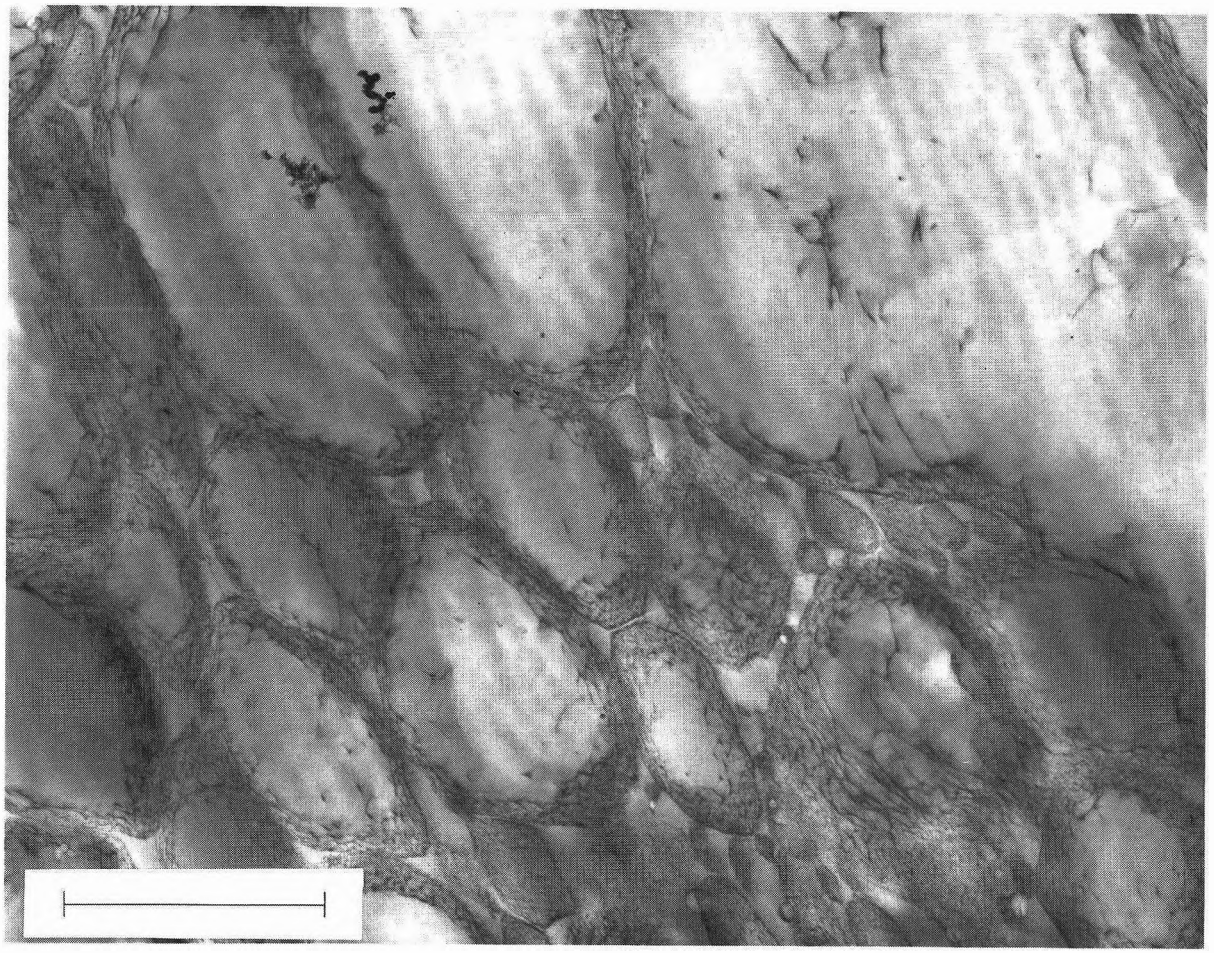

Figure 5. Cast, unleached, highly vulcanised PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

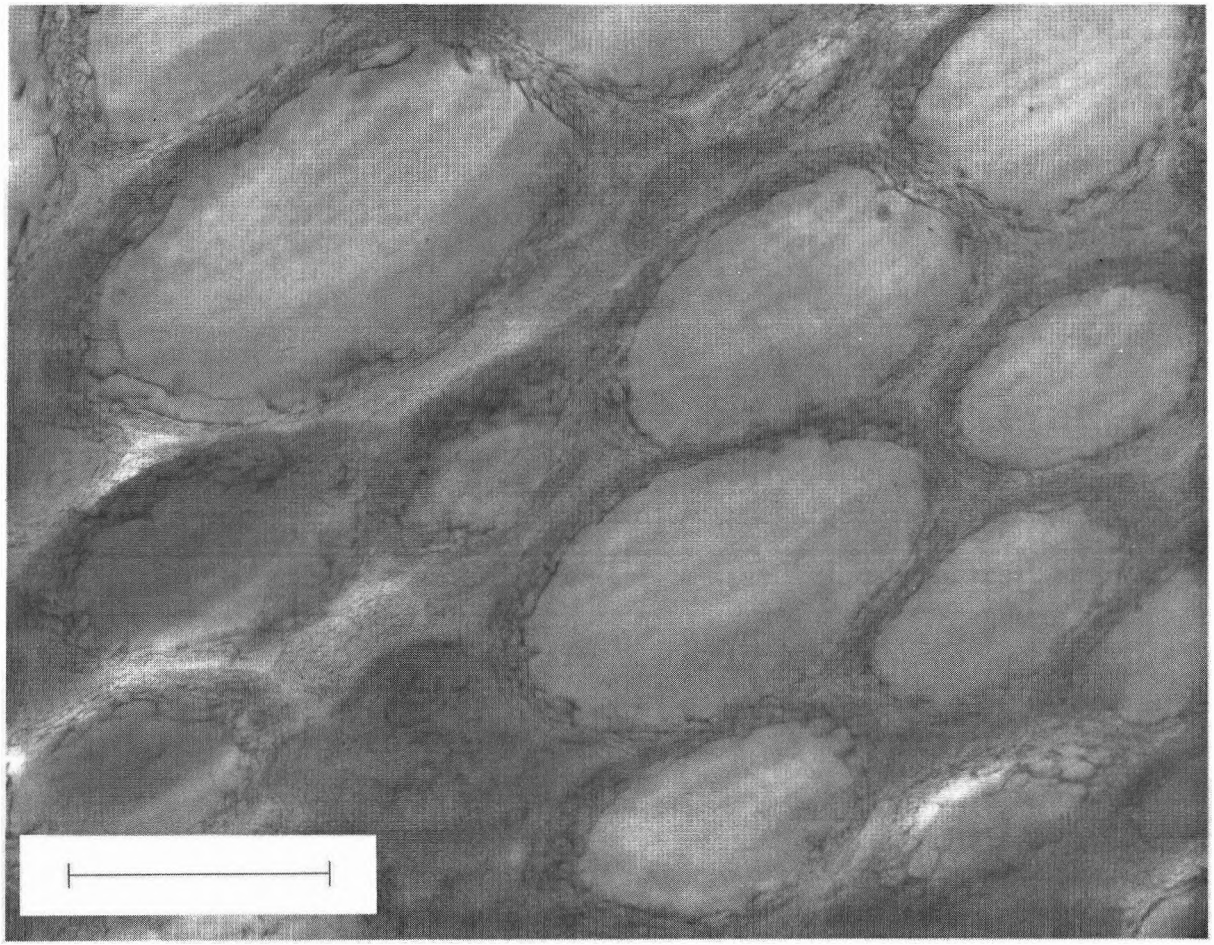

Figure 6. Cast, leached, highly vulcanised PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

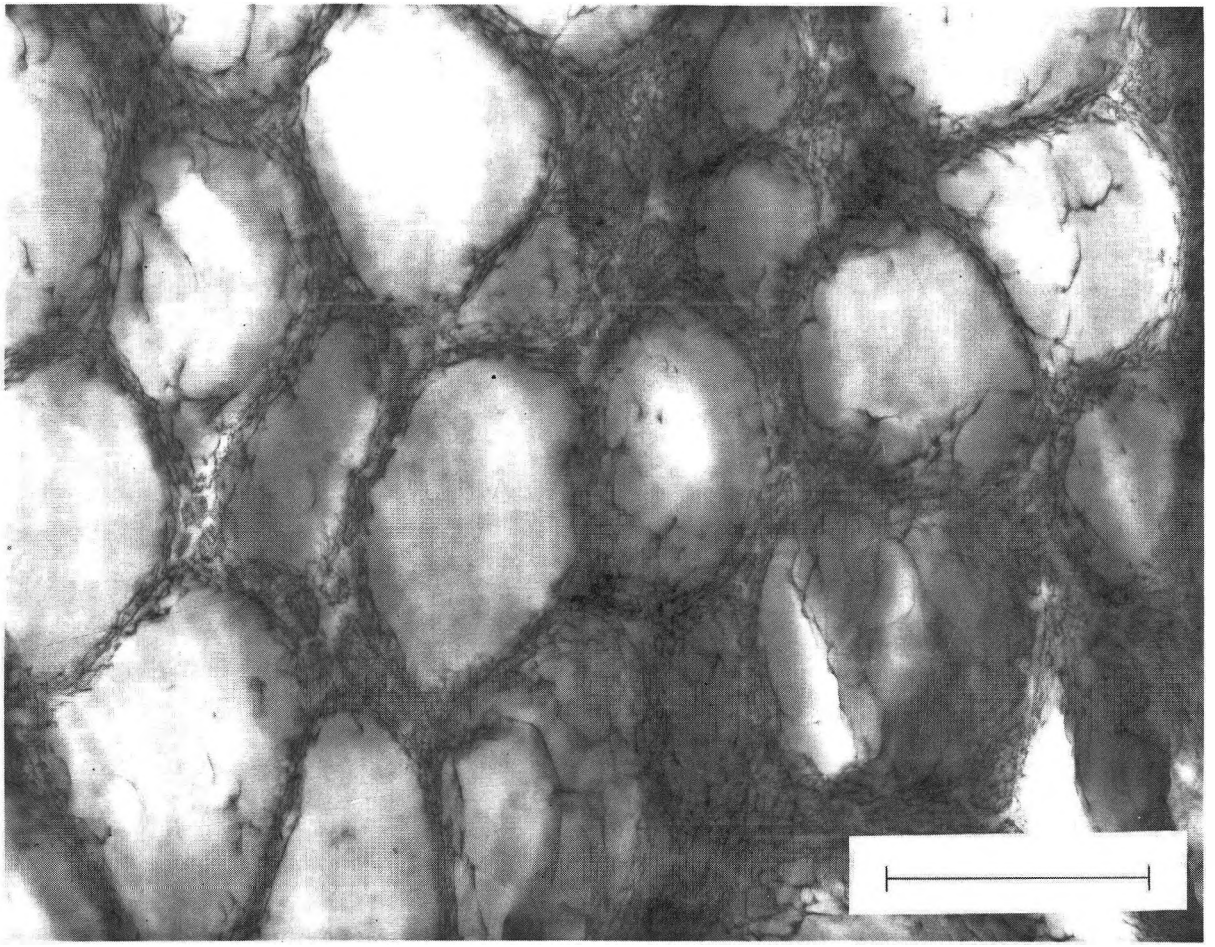

Figure 7. Cast, unleached CHP PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

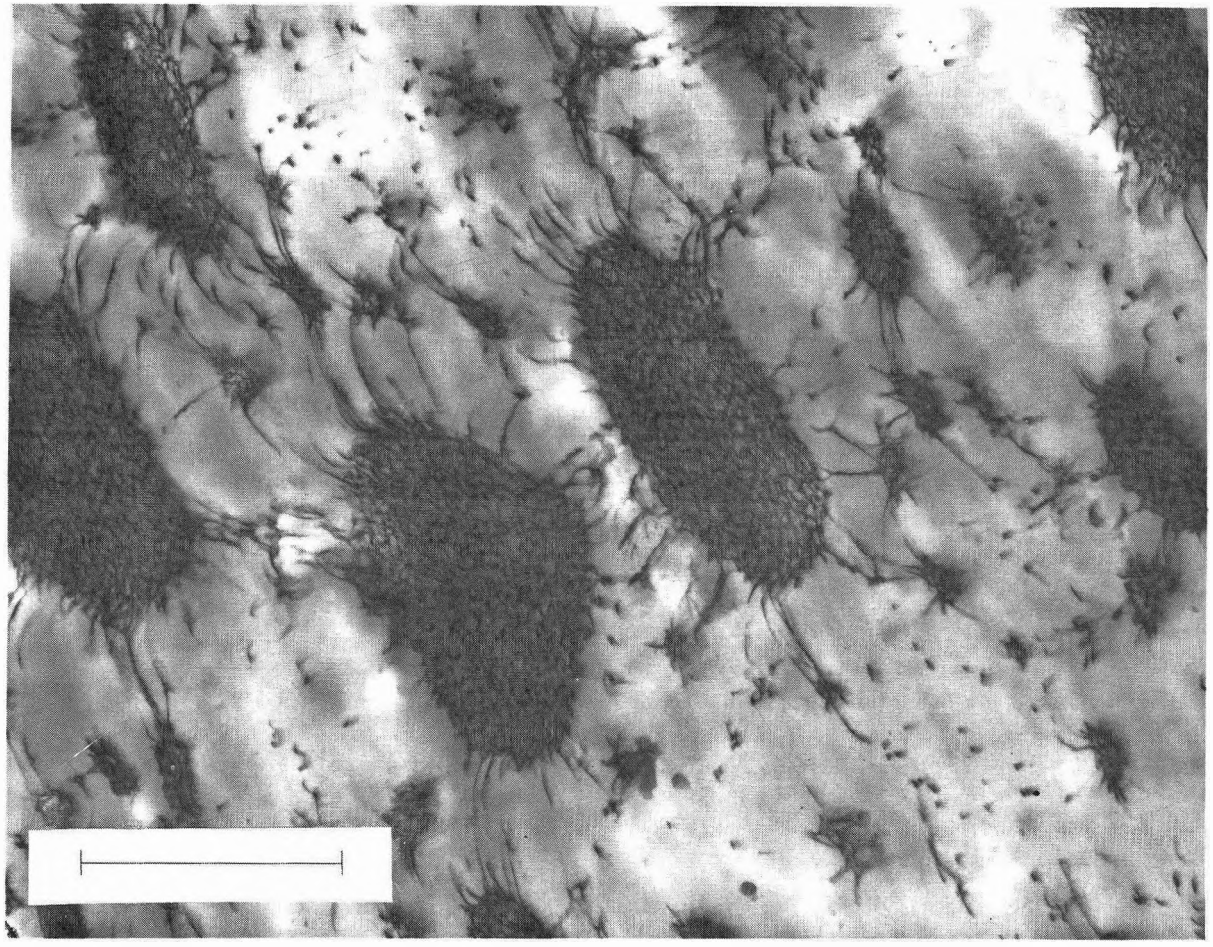

Figure 8. Cast, unleached DICUP PPVL film swollen with styrene, polymerised and stained with osmium tetroxide. Scale bar is 1 micron.

CONCLUSIONS

The technique of visualising the microstructure of latex films by styrene-swelling, polymerisation and osmium tetroxide staining has clearly revealed the inhomogeneous nature of crosslinking in peroxide prevulcanised latex particles. Only the small rubber particles are uniformly vulcanised (and these can have a very high crosslink density) while the large rubber particles are vulcanised only in a layer near their surfaces. This structure is likely to be found in any PPVL which is made by redox-activated processes in which the initiating radicals are generated in the aqueous phase.

Homogeneously crosslinked PPVL can be made by thermal activation of a peroxide which is completely dissolved in the rubber phase. However, in the case of dicumyl peroxide the reaction is too slow and inefficient to be of practical use. What would be required is a rubber-soluble peroxide which can be decomposed thermally at reasonable temperatures (less than 90°C) to produce radicals which are sufficiently active to cause crosslinking of the rubber within a few hours.

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Some Properties of Epoxidised Deproteinised Natural Rubber

A.H. ENG[*#], Y. TANAKA[**] AND S.N. GAN[***]

Some properties of epoxidised natural rubber (ENR) and epoxidised deproteinised natural rubber (EDPNR) were examined. It was found that DPNR latex reacts with peracetic acid in a similar manner as NR latex. Differences in the properties of modified rubbers are due to differences in the protein level of the raw materials used. Low gel content property of DPNR was also observed in the corresponding modified rubber, indicating that the processability of EDPNR was better than that of ENR. Epoxidation of rubber lattices does not cause much change in the non-rubber components of the rubbers.

Natural rubber is a useful 'green' raw material which has been widely used for more than a century. Chemical modification of natural rubber has been an area of interest for many rubber chemists because modified natural rubbers could have many potential commercial applications. Epoxidised natural rubber (ENR) is one such rubber which gives several improved properties in vulcanisates such as better oil resistance, resilience and lower gas permeation than those of natural rubber[1]. However, epoxidation of natural rubber leads to an increase in the gel content and hence reduces the processability of modified rubber[1]. In this study, the effects of epoxidation on some properties of deproteinised natural rubber (DPNR) such as gel content, density, ash content, acetone extract and glass transition temperature were investigated in comparison with that of ENR obtained from the same source.

MATERIALS AND METHODS

High ammonia (HA) latex, of dry rubber content 60%, prepared from fresh latex, was used for the preparation of ENR. The same source of latex was processed into DPNR latex of 60% dry rubber content.

The method of producing DPNR has been reported elsewhere[2]. This latex was then used to produce epoxidised DPNR.

Peracetic acid was freshly prepared by adding hydrogen peroxide to acetic anhydride by 40°C. The concentration of this acid was standardised by the method described by Greenspan and Mackeller[3].

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Reaction

Samples of NR latex were diluted with the same amount of water and stabilised with non-ionic surfactant, *Vulcastab LW* (from ICI Co., 1% w/v for HA latex and 0.5% w/v for DPNR latex). The pH of the diluted latices was adjusted to 6.0 by the addition of acetic acid. Appropriate amounts of freshly prepared peracetic acid were slowly added to the stirred latices which had been pre-cooled to 10°C. The mixtures were then allowed to react for 3 h at the same temperature. Upon completion of the reaction, the pH was adjusted to 7.1 and the rubber was coagulated by adding the latices to excess methanol with stirring. The rubbers were soaked overnight in water and dried at room temperature under reduced pressure.

Characterisation

For gel content analysis, about 0.5 g of each rubber was allowed to dissolve in 250 ml toluene without agitation in the dark for two weeks. The gel fraction was isolated by centrifugation at 11 000 r.p.m. (17 000 g) and dried at room temperature under reduced pressure to constant weight. The density of each rubber was measured by floatation method using methanol/water as the medium. Acetone extraction was carried out by soxhlet extraction of a 6 g rubber sample with acetone for 16 h in nitrogen atmosphere. Ash content was quantified by ashing 2 g of a rubber sample at 550°C for 8 h. ^{1}H-NMR measurements were performed by using 1% deuterated chloroform solutions of the rubber with TMS as an internal standard with a JEOL FX-100 NMR spectrometer. The sweep width was 1200 HZ, pulse delay 2.0 seconds and acquisition time 3.0 seconds. The peak areas were used to calculate epoxide content of the rubber by using the following equation:

$$\% \text{ Epoxide} = \frac{A_{2.7 \text{ p.p.m.}}}{A_{5.2 \text{ p.p.m.}} + A_{2.7 \text{ p.p.m.}}} \quad \ldots 1$$

where A is the area of the peak concerned.

The glass transition temperature (T_g) of each rubber was determined by a Seiko Instruments DSC 220 differential scanning calorimeter (DSC). Approximately 10 mg of a rubber sample, encapsulated in an aluminium sample pan, was heated to 80°C and quench-cooled by immersing the sample into liquid nitrogen. The sample was then inserted into the DSC at –140°C and scanned up to 50°C at a rate of 10°C/min. The mid-point value was used as T_g of the sample.

RESULTS AND DISCUSSION

Reaction of NR Latex and DPNR Latex with Peracetic Acid

The epoxidation reaction of NR latex with peracetic acid has been reported to be essentially quantitative. For reactions below ambient temperature and in the absence of strong acid catalysis, the side reactions are negligible[1,4]. This is also found to be the case for DPNR latex in the present study, as shown in *Figure 1*, where no signals due to ring-opened products such as furan and diol (3 p.p.m. – 4 p.p.m.) were detected by ^{1}H-NMR[5]. The epoxide contents of the modified NR and DPNR were determined by ^{1}H-NMR and summarised in *Table 1*.

The anticipated epoxide content was obtained based on the amount of standardised peracetic acid added to the latex, assuming a 100% conversion. Although a greater accuracy

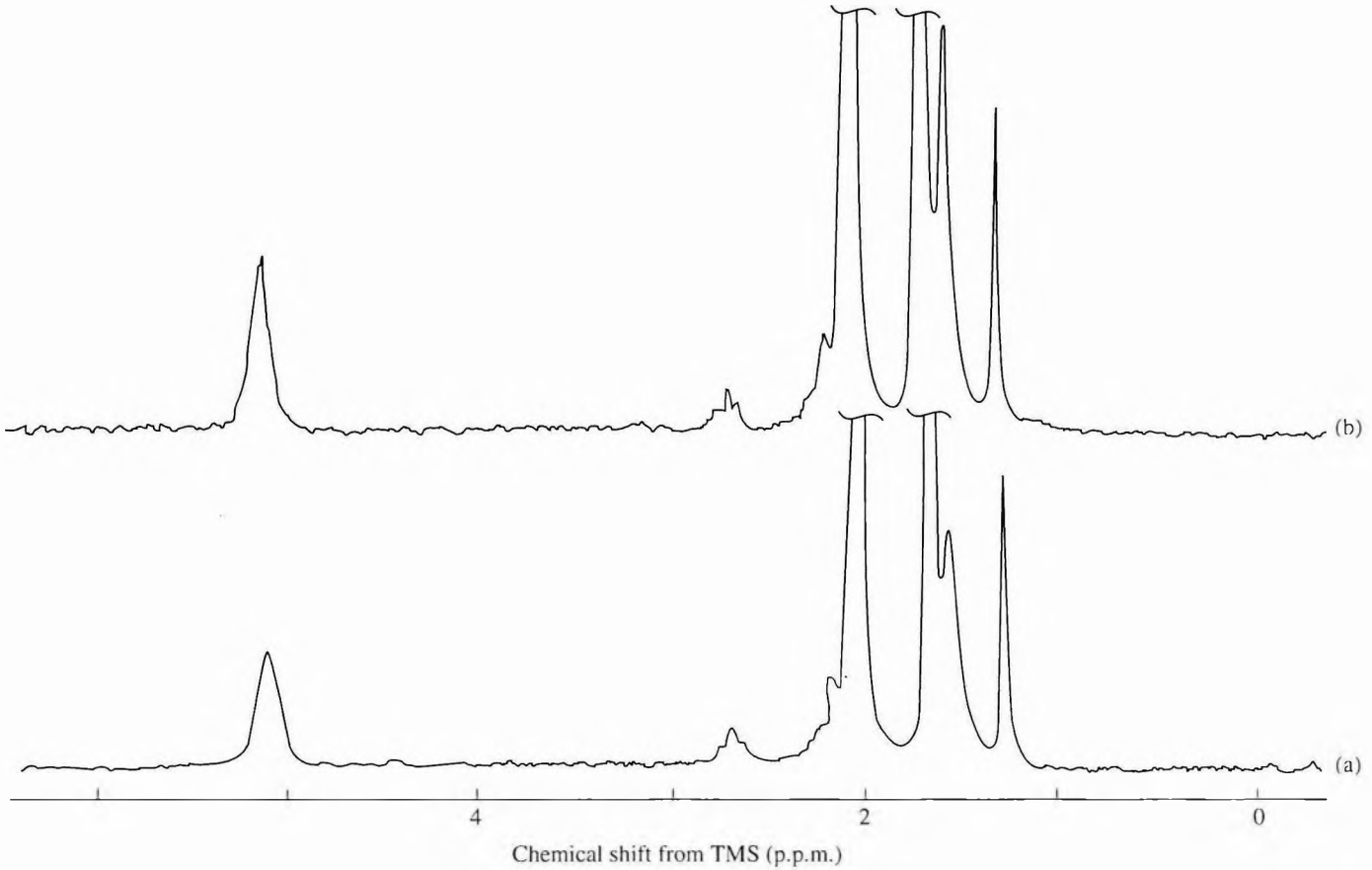

Figure 1. ^{1}H-NMR spectra of (a) 20% epoxidised natural rubber, and (b) 20% epoxidised deproteinised natural rubber.

TABLE 1. EPOXIDE CONTENT OF MODIFIED NR AND DPNR

Sample		Epoxide (anticipated)[a] (% mol)	Epoxide (^{1}H-NMR) (% mol)
1	ENR	13	11.0
2	EDPNR	13	11.5
3	ENR	25	21.8
4	EDPNR	25	21.6
5	ENR	50	48.8
6	EDPNR	50	51.1

[a]Calculated from the amount of peracetic acid added

of measuring the epoxide content could be obtained by hydrogen bromide (HBr) titration, the presence of gel in the sample, as shown in the later part of this paper, makes this difficult to be achieved. Data in *Table 1* indicate that NR and DPNR latices each gave a similar epoxide content, when the same amount of peracetic acid was added to the latex. This implies that the presence of proteins in natural rubber does not interfere with the formation of epoxide in latices.

These results, however, do not show the rate of epoxidation of each latex. Thus, the formation of epoxide, at different time intervals, was investigated. *Figure 2* shows that both NR and DPNR reacted at similar rates with peracetic acid, and that no induction period was observed. This probably implies that the size of peracetic acid molecule is small enough to diffuse through the protein layer surrounding the rubber particles as soon as it is added to the latex.

Gel and Nitrogen Content of ENR and EDPNR

The gel content of natural rubber has been reported to increase after epoxidation with peracetic acid[6]. This was also observed in the present study as shown in *Figure 3*. In the case of EDPNR, the increase in the gel content is less significant than in the case of ENR. This indicates that proteins play an important role in the gel formation in epoxidised natural rubber. This trend has also been observed in the case of natural rubber containing different amounts of protein[2]. For the level of epoxidation investigated, a small decrease in the protein content of the modified natural rubber with the level of modification was observed as indicated in *Table 2*. As substantial amounts of protein were still present in the modified NR as compared to DPNR, the increase in the gel content of ENR, therefore, is due to the interaction between epoxide groups and rubber proteins.

Gel in ENR has been reported to reduce the processability of the modified rubber. In this study, EDPNR was found to contain lower amounts of gel than ENR, implying that the processability of EDPNR is better than that of ENR.

Ash Content

The ash is the mineral component of natural rubber, which is held by polar non-rubber

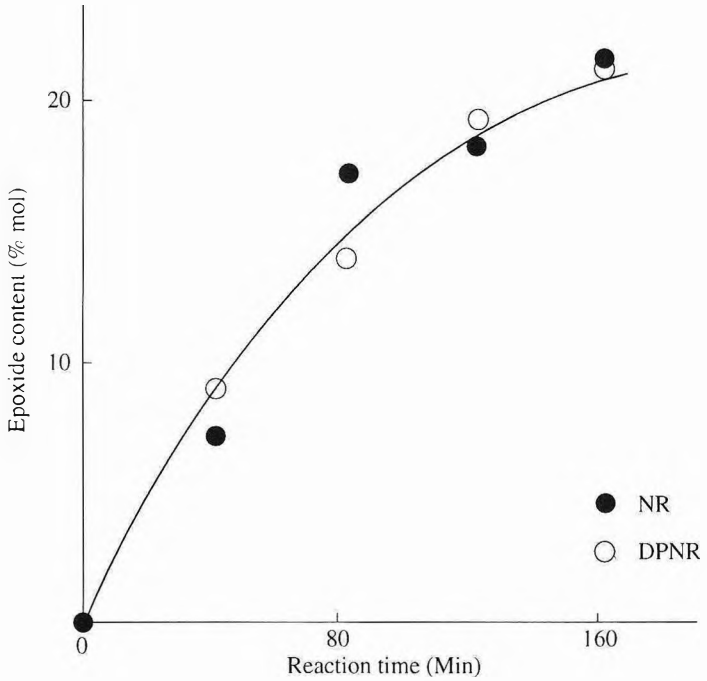

Figure 2. Formation of epoxide at different time intervals.

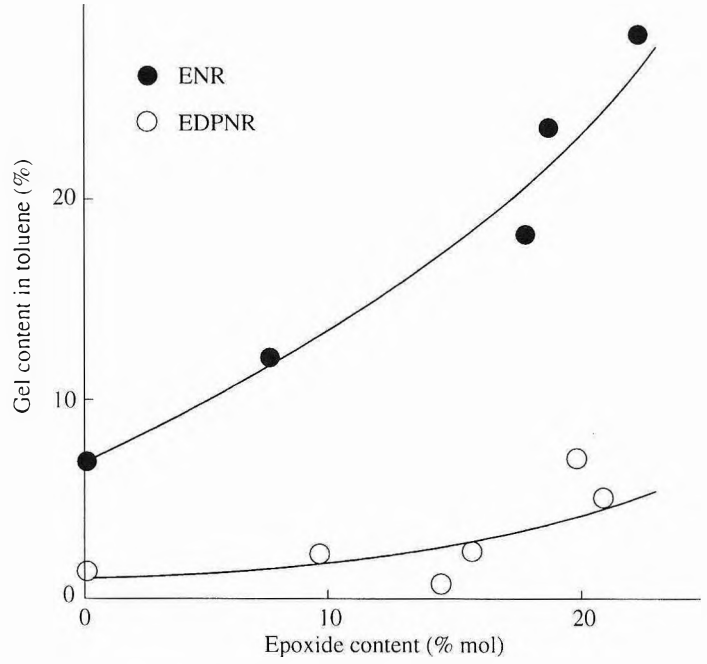

Figure 3. Gel content of modified natural rubbers.

TABLE 2. NITROGEN CONTENT OF NR AND DPNR BEFORE AND AFTER EPOXIDATION

Epoxide content (% mol)	Nitrogen content (% wt) NR	DPNR
0	0.18	0.01
22	0.17	0.01
50	0.14	0.01

components in natural rubber such as proteins and lipids. Therefore, in the absence of rubber proteins, DPNR is expected to contain a reduced ash content and this was found to be the case in the present study, as indicated in *Table 3*.

TABLE 3. ASH CONTENT OF NR AND DPNR BEFORE AND AFTER EPOXIDATION

Epoxide content (% mol)	Ash content (% wt) NR	DPNR
0	0.23	0.12
22	0.19	0.12
35	0.21	0.13
50	0.18	0.11

Upon epoxidation, the ash content of both rubbers showed insignificant changes, probably because most of the polar non-rubber components, such as proteins which hold the mineral components of natural rubber[7], were still present in the modified rubber.

Acetone Extract

Extraction of natural rubber with acetone removes resins such as phenolic compounds, colouring materials, free fatty acids and their esters. There is no significant difference between the amount of acetone extract from NR and DPNR as shown in *Table 4*.

TABLE 4. ACETONE EXTRACT OF NR AND DPNR BEFORE AND AFTER EPOXIDATION

Epoxide content (% mol)	Acetone content (% wt) NR	DPNR
0	2.2	2.2
22	2.1	2.3
50	2.4	2.5

Epoxidation of NR and DPNR did not change the amount of acetone extract in the modified rubbers; this implies that peracetic acid does not change the solubility of non-rubber components in acetone.

Glass Transition Temperature

Continual epoxidation of polydienes invariably leads to an increase in the T_g value because the presence of bulky epoxide group in the polymer main-chain lowers rotational freedom of the modified segment. In the present study, the T_g's of the quenched amorphous polymers are indeed found to increase in step with the level of modification, as shown in *Figure 4*. Both ENR and EDPNR showed an increase of 0.82 K per mol% in their T_g's which is quite similar to the reported value of 0.85 K per mol%[8]. These results indicate that rubber proteins have no significant effect on the segmental mobility of both *cis* polyisoprene and partially epoxidised *cis* polyisoprene. This has also been confirmed in a study on the thermal properties of DPNR[9].

Density

Purification of natural rubber is expected to affect the density of the polymer. *Figure 5*

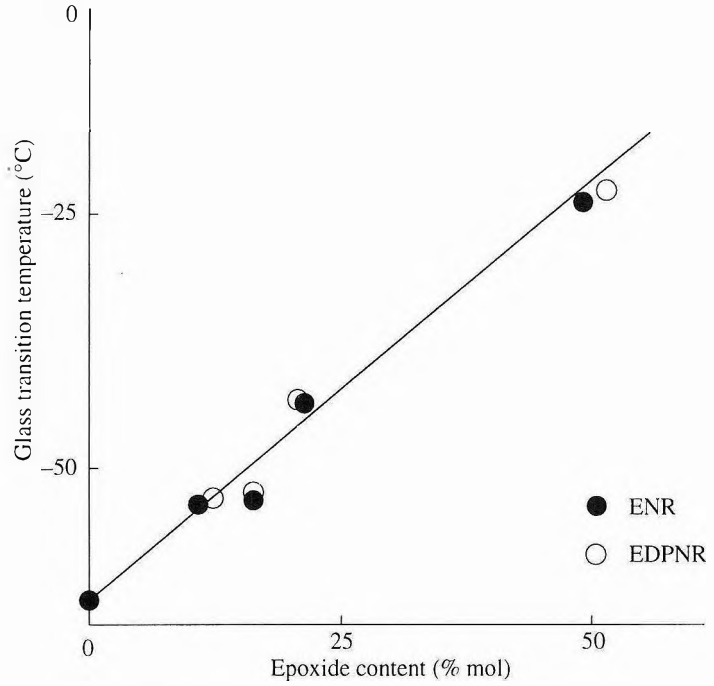

Figure 4. Glass transition temperature of modified natural rubbers.

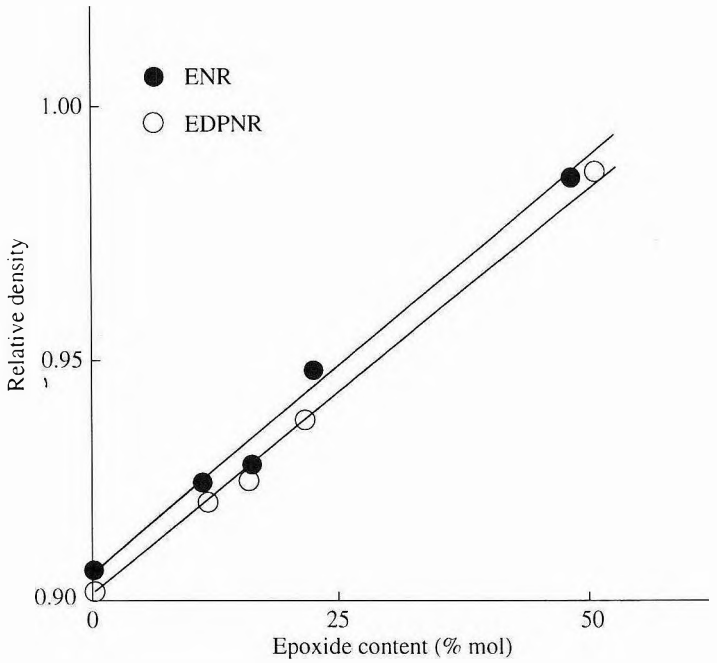

Figure 5. Density of modified natural rubber.

indicates that the density of NR is slightly higher than that of DPNR. This is not unexpected because DPNR contains less rubber proteins and ash, both of which have higher density than the rubber hydrocarbon. It has been reported that the density of epoxidised NR increases with the epoxide content[4]. In the present study, as the level of epoxidation of NR and DPNR increases, the density of both rubbers increases in a parallel manner. Epoxidation does not change the non-rubber composition of the modified rubber very much. Therefore, the difference between the densities of ENR and EDPNR of the same epoxide content is very similar to that between NR and DPNR.

CONCLUSION

Although there are several differences between properties of ENR and those of EDPNR, such as ash and gel contents, epoxidation does not change the non-rubber content of the modified rubbers very much. The differences are therefore derived from the raw materials used to produce the modified rubbers. Deproteinisation of NR before epoxidation lowers the gel content and therefore improves the processability of the modified rubber.

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High Damping NR/Epoxidised NR Blends

P.S. BROWN* AND A.J. TINKER*#

High damping blends possessing good physical properties are obtained when a lightly filled NR compound is blended with a heavily filled and plasticised epoxidised natural rubber (ENR) compound. The use of a polar plasticiser ensures that the plasticiser is distributed in favour of the more polar ENR phase. Damping is found to vary with the black distribution, being favoured by a large difference between the loading in each phase. The level of damping increases with the proportion of the ENR component in the blend, but the other physical properties worsen. Recovery properties are poor for all blends except those vulcanised by using an efficient vulcanisation cure system. The high level of damping that these materials possess results from the high filler and plasticiser loading in the ENR phase, however, the relatively high T_g *of this component influences how the properties vary with changing temperature. The dynamic loss peak at 5 Hz lies just below 0°C for blends based on ENR-50, and at about –31°C for the blends containing ENR-25. A marked stiffening accompanies this glass transition, limiting the operating range of the materials. ENR-50 based blends give higher damping but have more temperature dependent properties when compared to their ENR-25 analogs.*

Vulcanised rubbers are visco-elastic materials which exhibit the property of hysteresis under dynamic stresses. This energy absorption property is utilised in vibration isolation[1] for example in automotive bushes and mounts or in building bearings for earthquake protection[2]. The amount of energy loss, or damping, is dependent on the temperature and its relationship to the rubber's glass transition temperature (T_g), and also on the formulation of the compound. High damping materials are usually produced using either high T_g rubbers[3] or by using a combination of high filler and plasticiser loadings in a rubber with a low T_g, usually in conjunction with a low curative level[4]. Both approaches result in some property inadequacies; using a high T_g polymer means that the temperature dependence of the dynamic properties is quite large, whilst highly filled and plasticised materials usually possess poor ultimate properties.

A blend between a highly filled and plasticised rubber and a more normally compounded rubber might offer a material with both high damping and good strength properties. However, the choice of plasticiser and polymers is very important since diffusion will occur to produce the equilibrium distribution of plasticiser in the blend, so the plasticiser must have a large partition coefficient in favour of one of the polymers, the highly filled polymer.

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This approach has been demonstrated for blends of highly filled epoxidised natural rubber (ENR-50, 50 mol% epoxidation) and natural rubber (NR)[5]. Diffusion of the plasticiser away from the highly filled ENR-50 phase was avoided by using a polar plasticiser. The equilibrium distribution of such a plasticiser is biased towards the ENR-50 phase as this is the more polar elastomer. Aris[6] used a polymeric ester plasticiser with a distribution coefficient (Kd) in favour of the ENR-50 of 5.35. Although ENR-50 has a high T_g and thus high intrinsic damping[3], it is not this property of the ENR-50 that is important in these particular blends, but the high polarity that promotes the uneven distribution of the polar plasticiser. A high plasticiser loading allows a high filler content in the ENR phase, and these combine to provide the high level of damping.

This study extends the understanding of these new materials by investigating the effect of some of the compounding variables on material properties.

MATERIALS AND METHODS

The rubbers used in this study were natural rubber, NR (SMR CV), 50mol % epoxidised NR, ENR-50 (*Epoxyprene 50*, Guthrie, Malaysia) and 25mol % epoxidised NR, ENR-25 (*Epoxyprene 25*, Guthrie, Malaysia). Rubber chemicals and fillers were standard commercial grade materials; the plasticisers were tributoxyethyl phosphate, TBEP (*Amgard TBEP*, Albright & Wilson, UK) and an alcohol modified polymeric resin plasticiser, *Diolpate 7017* (Kemira Polymers, UK).

Compounding was performed using a BR size Banbury internal mixer. All of the blend materials are produced by crossblending well mixed single polymer masterbatches. This was to ensure that the loading of carbon black in the individual phases of the blend is both known and controlled. Both NR and ENR interact strongly with carbon black and the masterbatches were well mixed; this should ensure that little or no migration of filler occurred during crossblending[7]. To remove absorbed water from the carbon black it was dried at 125°C for 2 h prior to use. This technique was found to minimise black losses during mixing. The single polymer masterbatches were produced using the mix cycles given in *Table 1*, the mix cycle for the NR component is slightly longer so as to give mixes of similar Mooney viscosity (M_L 1 + 4 at 100°C). The large quantities of oil and plasticiser in the ENR phase require a two stage addition of both carbon black and plasticiser and careful control of the ram position and pressure to avoid excessive material losses. Crossblending was performed in the Banbury mixer at a rotor speed of 116 r.p.m. for 2 min, or, for small scale mixes, on a cool two roll mill. The curatives were added on a cool two roll mill. The ENR mixes and the blends show a tendency to band on the back (higher speed) mill roller during milling.

Test pieces were vulcanised at 150°C in a steam heated press to t_{max} as determined by using Monsanto ODR or MDRE rheometers. Metal-rubber bonding of the double shear test pieces used a two-coat system (*Chemlok 205* primer and *Chemlok 220* top coat). These samples were vulcanised by using cold metal pieces in the pre-heated transfer mould and extending the vulcanisation period by 5 min.

The physical properties were obtained by using the relevant international test. The dynamic properties in double shear test

TABLE 1. SINGLE POLYMER MASTERBATCH MIX CYCLES

Time	ENR masterbatch	NR masterbatch
Start	Polymer + calcium stearate	Polymer
30 s	Powders + 1/2 black + 1/4 plasticiser	Powders + 1/2 black
1 1/2 min	1/2 black + 3/4 plasticiser[a]	1/2 black + plasticiser
2 1/2 min	Sweep	Sweep
4 min	Dump	
4 1/2 min		Dump

[a]Ram to neutral for ~ 15 s as plasticiser is absorbed

geometry were determined by using an Instron 1271 test machine. This instrument is actuated by a servo-hydraulic drive and is fitted with an environmental chamber. Test data were collected by using a Schlumber Solartron 1250 frequency response analyser. Room temperature testing was performed on samples conditioned by using a set pre-test strain sequence of 3 cycles of 0%–100% followed by 3 cycles of 0 – ±100% at a frequency of 1 Hz–2 Hz. Testing commenced without further delay, beginning at small strains (0.1%) and increasing strain in stages to a maximum of 20%. The test frequency was 5 Hz.

The temperature sweep tests were performed by first cooling the chamber and sample to approximately – 40°C. This temperature was maintained for 30 min prior to testing to allow thermal equilibrium to be reached. The temperature of the test piece was monitored by use of a thermocouple wire fixed to one of the metal ends of the test piece. The samples were very stiff at this low temperature; strains in excess of 1% caused the instrument overload protection system to function, hence the use of this lower strain for the sweep. The temperature was allowed to increase to room temperature over a period of 2 h–3 h, during which time the test data were obtained. The chamber was then heated in small steps to 40°C, allowing 10 min–20 min for thermal equilibration to occur at each intermediate temperature. Samples only experienced the dynamic strains during the acquisition of the individual datum points.

RESULTS AND DISCUSSION

Effect of Carbon Black Distribution

In his work, Aris[5] used a loading of 50 p.h.r. carbon black in the NR phase and 90 p.p.h.r. carbon black in the ENR-50 phase. The same grade of carbon black was used in the two phases. The loadings of the polymeric ester plasticiser were 5 p.h.r. and 40 p.h.r., respectively. To achieve such a disparate black distribution the blend was produced by crossblending two separately mixed single polymer masterbatches.

A study to determine the effect of the black distribution on the dynamic properties of the

blends was conducted by crossblending single polymer masterbatches of ENR-50 and NR at a polymer ratio of 1:1. As with the earlier work the same carbon black was used in both polymers. Two ENR-50 and three NR masterbatches were produced (1, 2 and 4 – 6, *Table 2*). Five compounds (7–11, *Table 3*) were produced by crossblending these masterbatches at a 1:1 polymer ratio and finalising using a sulphur-CBS semi-EV cure system; two analogous single polymer vulcanisates (12 and 13) were also produced (*Table 3*). It is evident that the blends show the high damping of the ENR component. Damping is greatest when the difference in black loading in the two phases is greatest (entries 7 and 8 or 9 and 10, *Table 4*). Damping increases with both increased loading in the ENR phase and with decreased loading in the NR phase. The blends show a level of damping that is greater than the simple mean of the two component values, suggesting that the ENR phase is continuous. Obtaining micrographs to support this thesis proved impossible due to the black loadings defeating attempts to obtain contrast between the two phases.

A second set of blends with a higher loading of carbon black in the ENR-50 was used to investigate the effect of cure system (Compound 3, *Table 2*). A range of curative loadings within the semi-EV cure system employed by Aris[5] and a fully efficient cure system were used to produce these blends (*Table 5*). The static physical properties of these blends are generally good (*Table 6*), the tensile strengths are close to 20 MPa whilst the extensions at break are greater than 500%. The semi-EV cure system gives poor compression set at 70°C (> 40%), that of the EV cure is considerably better.

Changing the polymer ratio could change the blend morphology, and thus the properties of the blend. Blends were produced at 1:1 and 6:4 volume ratios (NR:ENR masterbatches, *Tables 7* to *9*). As expected, the loss angle is reduced as the proportion of the NR masterbatch is increased (also compare blend 16, *Table 6* with blends 21 and 22, *Table 9*). Care must be taken in interpreting the dynamic data as the loss angle has an inverse relationship with the dynamic modulus, however, the EV vulcanisates appear to be giving higher loss angles. The compression set of the blends cured using the conventional and semi-EV cure systems was poor (*Table 9*), whilst that of the EV system blends was good. The TMTD/TBBS system was shown by Gelling and Metherell to give ENR vulcanisates with good compression set[8]. The tensile properties of blends 25–28 were inferior to those of the other blends; to maximise the tensile strength the proportion of the NR masterbatch needs to be high.

Temperature Variation of Dynamic Properties

The use of the high T_g ENR-50 in these blends is likely to confer a high rate of change on the dynamic properties of the material with temperature, particularly as the temperature is reduced below ambient. This is evident in *Figure 1*, which shows the ratio of the dynamic modulus (G') at a given temperature to that at 23°C. Blend 28 (▼, *Figure 1*) shows a significant stiffening even at 0°C, with a seven-fold increase in dynamic modulus at –10°C and a twelve-fold increase at –20°C. These changes are accompanied by changes in loss angle (*Figure 2*), the loss peak is at –2°C and the loss tangent falls rapidly with temperature below this point, being only a third of the room

TABLE 2. SINGLE POLYMER MASTERBATCH FORMULATIONS I

Mix Number	Formulation 1	2	3	4	5	6
SMR CV				100	100	100
ENR-50	100	100	100			
N 330 BLACK	90	100	110	30	45	55
Diolpate 7017	40	40	40	5	5	5
Zinc oxide	5	5	5	5	5	5
Stearic acid	2	2	2	2	2	2
TMQ[a]	2	2	2	2	2	2
Calcium stearate	3	3	3			
Mix wt.	242	252	262	144	159	169
Mix vol. (cm^3)	194.3	199.9	205.4	136.6	143.9	149.5
Specific gravity	1.245	1.261	1.275	1.062	1.105	1.13

[a]Poly-2,2,4-trimetyl-1,2-dihydroquinoline *e.g. Felectol H* (Monsanto)

TABLE 3. CROSSBLEND FORMULATIONS I

Masterbatch	Formulation 7	8	9	10	11	12	13
4					67		
1	121		121				242
2		126		126	126		
5	79.5	79.5				159	
6			84.5	84.5			
Polymer ratio	1:1	1:1	1:1	1:1	1:1		
Mix volume ratio	4:6	4:6	4:6	4:6	4:6		
Sulphur	1.2	1.2	1.2	1.2	1.2	1.2	1.2
CBS[a]	1.2	1.2	1.2	1.2	1.2	1.2	1.2

[a]N-Cyclohexyl-2-benzthiazole sulphenamide

TABLE 4. DYNAMIC PROPERTIES (10% STRAIN, 5 Hz AT 23°C)

Dynamic properties	Formulation						
	7	8	9	10	11	12	13
Black ratio[a]	45:90	55:90	30:100	45:100	55:100	45	90
G'(MPa)	2.44	2.22	2.19	2.69	2.54	1.47	3.71
Loss angle,	19.3	18.6	19.8	19.5	19.3	8.26	22.9
Tan(δ)	0.350	0.337	0.360	0.354	0.350	0.145	0.422

[a]NR: ENR, p.h.r. black in each phase. Black loading in the case of single polymer vulcanisates 12 and 13

TABLE 5. CROSSBLEND FORMULATIONS II

Masterbatch	Formulation				
	14	15	16	17	18
3	131	131	131	131	131
4	72	72	72	72	72
Polymer ratio	1:1	1:1	1:1	1:1	1:1
Mix volume ratio	4:6	4:6	4:6	4:6	4:6
Sulphur	0.75	1	1.2	1.5	0.4
CBS[a]	0.75	1	1.2	1.5	6

[a]N-Cyclohexyl-2-benzthiazole sulphenamide

temperature value at –20°C. A plasticiser with a lower T_g would reduce the temperature dependence of the properties of a single polymer ENR vulcanisate, but in these blends the plasticisers were selected primarily on the basis of their partition between the two polymers, a criterion which greatly reduces the number potential plasticisers.

Perceived applications for these compound require the component to operate over a temperature range of –20°C to 30°C, for example as automotive damping materials for use in northern Europe or northern America. The ENR-50 T_g, and thus the change in material properties, occurs at a temperature which is well within this range. Thus the NR/ENR-50 blends may not be suitable for use as high damping materials in these regions. ENR-25 is the 25mol % epoxidised analogue of ENR-50 and therefore has a lower T_g[9]. Blends of NR with ENR-25 would be expected to have a dynamic loss peak at a lower temperature than the analogous NR/ENR-50 blends. The lower level of epoxidation results in a lower polarity polymer and this reduces the plasticiser Kd to

TABLE 6. PHYSICAL PROPERTIES

Properties	Formulation 14	15	16	17	18
Cure system[a]	SEV	SEV	SEV	SEV	EV
Tensile strength (MPa)	16.8	18.8	19	21	18.1
Extension at break (%)	595	570	540	505	525
M300 (MPa)	8.15	10	10.9	12.9	9.92
MR 100 (MPa)	1.28	1.72	1.97	2.52	1.7
Hardness (IRHD)	66	70	71	75	73
Compression set (1 day at 70°C)	57	54	52	44	26
Compression set (3 days at 23°C)		27	22	19	25
G' (MPa[b])	2.14	2.21	2.45	3.08	2.31
Loss angle	22.5	22.3	21.9	20.3	23.4
Tan(δ)	0.414	0.41	0.402	0.37	0.433

[a]SEV: semi-EV cure system, accelerator: sulphur ratio ~ 1:1; EV: efficient cure system accelerator: sulphur ratio ~ 5:1

[b]Dynamic properties determined at 10% strain, 5 Hz at 23°C.

TABLE 7. SINGLE POLYMER MASTERBATCH FORMULATIONS II

Mixes	Formulation 19	20
SMR CV	100	
ENR-50		100
N 330 BLACK	30	110
Diolpate 7017	6	45
Zinc oxide	5	5
Stearic acid	2	2
TMQ[a]	2	2
Calcium stearate		3
Mix wt.	145	267
Mix vol. (cm³)	136.6	210.4
Specific gravity	1.061	1.269

[a]Poly-2,2,4-trimetyl-1,2-dihydroquinoline *e.g.*, *Felectol H* (Monsanto)

3.82[6], which will mean that the degree of plasticisation of the ENR phase is reduced.

The ENR-25 blend analogous to 26 (*Table 9*) was prepared and found to bleed the *Diolpate* plasticiser on storage. Reducing the *Diolpate* level in the blend may alleviate this problem, but additional plasticisation of the ENR-25 would then be required. TBEP has a much lower T_g than *Diolpate 7017* (−114°C *vs.* −88°C) but similar Kd between NR and ENR-25[6]. A partial substitution of *7017* with TBEP was used for the ENR-25 blends giving a mix with a slightly reduced plasticiser loading to offset the better plasticisation of the TBEP (blend 30, *Table 10*). The TBEP was compounded into the ENR masterbatch, but some will diffuse into the NR component during crossblending. Three levels of curative

TABLE 8. CROSSBLEND FORMULATIONS III

Masterbatch	Formulation							
	21	22	23	24	25	26	27	28
19	88.5	101.5	88.5	101.5	88.5	101.5	88.5	101.5
20	104	80.1	104	80.1	104	80.1	104	80.1
Polymer ratio	61:39	70:30	61:39	70:30	61:39	70:30	61:39	70:30
Mix volume ratio	1:1	6:4	1:1	6:4	1:1	6:4	1:1	6:4
Sulphur	1.2	1.2	2	2	0.2	0.2	0.3	0.3
CBS[a]	1.2	1.2	0.4	0.4			4.5	4.5
TMTD[b]					1.3	1.3		
TBBS[c]					2	2		

[a]N-Cyclohexyl-2-benzthiazole sulphenamide

[b]Tetramethylthiuram disulphide

[c]N-tert-Butyl-2- benzothiazylsulphenamide

TABLE 9. PHYSICAL PROPERTIES II

Properties	Formulation							
	21	22	23	24	25	26	27	28
Blend mix volume ratio	1:1	6:4	1:1	6:4	1:1	6:4	1:1	6:4
Cure system	SEV	SEV	CON	CON	EV	EV	EV	EV
Tensile strength (MPa)	20.3	22.5	14.8	16.5	18	20	16.7	19.2
Extension at break (%)	550	580	470	560	570	580	600	630
M 300 (MPa)	10.2	10.2	9.07	7.66	8.93	8.79	7.13	7.11
MR 100 (MPa)	1.96	1.88	1.82	1.46	1.74	1.63	1.29	1.23
Hardness (IRHD)	67	63	67	62	65	64	61	58
Compression set (1 day at 70°C)	38	32	40	38	24	22	24	23
Compression set (3 days at 23°C)	18	18	23	21	22	21	23	21
G' (MPa[a])	1.69	1.58	1.49	1.46	1.69	1.53	1.53	1.42
Loss angle	22.1	19.6	24.4	21.9	24	22	23.7	22.8
Tan (δ)	0.407	0.357	0.454	0.402	0.445	0.405	0.44	0.421

[a]Dynamic properties determined at 10% strain, 5 Hz at 23°C

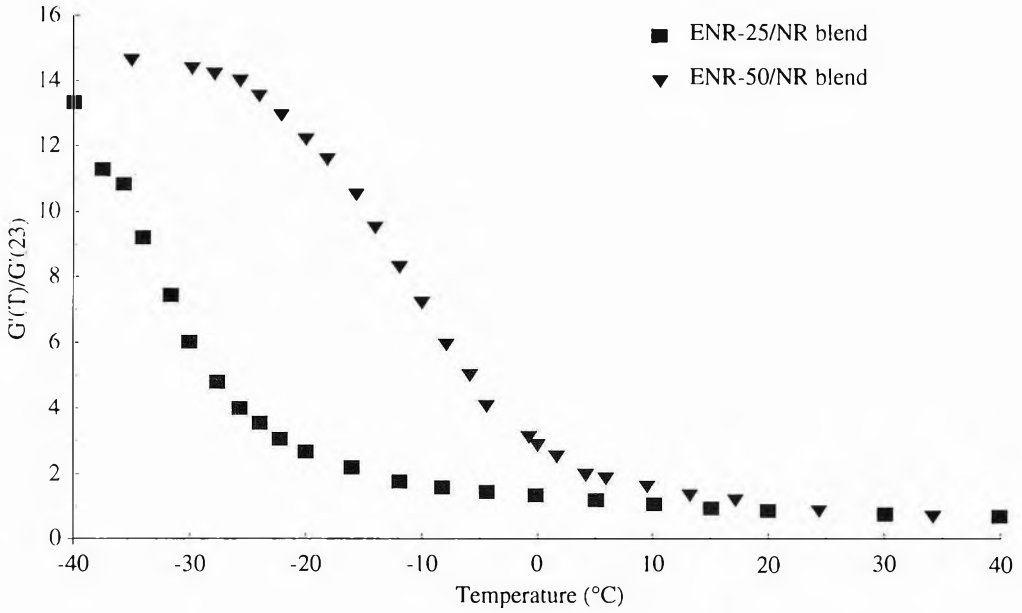

Figure 1. Instron DMTA of ENR-50 and ENR-25 blends: Change of real modulus with temperature at 5 Hz and 1% strain, normalised to 23°C.

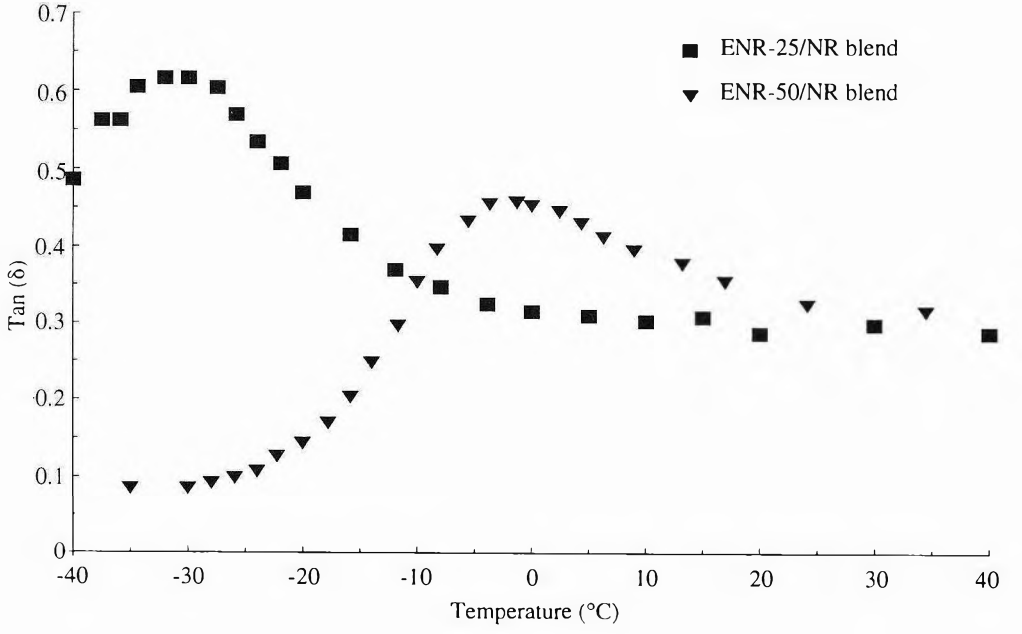

Figure 2. Instron DMTA of ENR-50 and ENR-25 blends: Variation of tangent (δ) with temperature at 5 Hz and 1% strain.

TABLE 10. SINGLE POLYMER MASTERBATCH FORMULATIONS III

Mixes	Formulation 29	30
SMR CV	100	
ENR-25		100
N 330 BLACK	30	110
Diolpate 7017	6	30
Zinc oxide	5	5
Stearic acid	2	2
TMQ[a]	2	2
Calcium stearate		3
TBEP		12
Mix wt.	145	264
Mix vol. (cm^3)	136.6	207.4
Specific gravity	1.061	1.273

[a]Poly-2,2,4-trimetyl-1,2-dihydroquinoline *e.g. Felectol H*

were used (*Table 11*), these gave hardness values ranging from 49 IRHD to 55 IRHD, tensile strengths of at least 19 MPa, but somewhat lower loss angles than the ENR-50 analogue at about 18° (blend 33, *Table 12 vs.* Blend 26, *Table 9*). Dynamic stiffness and compression set were also lower for blend 33, the ENR-25 blend. No plasticiser bleeding was observed up to six months after vulcanisation.

The lower T_g of the ENR-25, together with a possible influence of the lower T_g of the TBEP, does provide a solution to the temperature dependence of the properties. The increase in dynamic modulus is moved along the temperature axis by about –20°C (■, *Figure 1*), the loss angle peak occurring at –31°C (■, *Figure 2*). The temperature dependence of both of these properties is very low over the range 40°C – 15°C, but does increase below this temperature. However, even at –30°C the increase in dynamic stiffness over the room temperature value is only by a factor of 6. The ENR-25 blends would appear to offer a solution for low temperature applications, albeit with slightly lower damping.

CONCLUSIONS

Blends of normally compounded natural rubber compounds with highly filled and plasticised epoxidised natural rubber compounds produce materials which possess good physical properties, particularly tensile and recovery properties, yet give high levels of damping. The potential for plasticiser diffusion out of the ENR phase is avoided by the use of polar plasticisers with high affinities for the ENR phase. The relatively high T_g of ENR-50 means that its blends show a quite marked temperature dependence of the dynamic properties below ambient temperatures, but give high loss angles and good strength properties. A reduction in the temperature dependence is found for blends of ENR-25 with NR, although this is accompanied with a reduction in the level of damping. High damping is favoured by a large difference in the black loading of the two polymers and by a high proportion of ENR in the blend. This latter factor gives lower ultimate strength properties.

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TABLE 11. CROSSBLEND FORMULATIONS IV

Masterbatch	Formulation 31	32	33
29	101.5	101.5	101.5
30	79.2	79.2	79.2
Polymer ratio	70:30	70:30	70:30
Mix volume ratio	6:4	6:4	6:4
Sulphur	0.13	0.16	0.19
TMTD[a]	0.85	1.04	1.24
TBBS[b]	1.3	1.6	1.9

[a]Tetramethylthiuram disulphide

[b]N-tert-Butyl-2-benzothiazylsulphenamide

TABLE 12. PHYSICAL PROPERTIES II

Properties	Formulation 31	32	33
Tensile strength (MPa)	19	21.5	22.3
Extension at break (%)	715	665	640
MR 100 (MPa)	0.962	1.28	1.4
Hardness (IRHD)	49	53	55
Compression set (1 day at 70°C)	26	21	18
Compression set (3 days at 23°C)	20	14	12
G' (MPa)[a]	1.04	1.1	1.18
Loss angle	18.5	18	17.7
Tan(δ)	0.335	0.326	0.32

[a]Dynamic properties determined at 10% strain, 5 Hz at 23° C

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J. nat. Rubb. Res., **12(1)**, 102–111

A Guide to Identifying Common Inorganic Fillers and Activators Using Vibrational Spectroscopy

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Vibrational spectroscopy, (FT-IR and FT-Raman spectroscopy) has been used to study a range of inorganic fillers and activators. The paper demonstrates the information available from vibrational spectroscopy and suggests areas in which useful data can be gained.

Three specific examples of the use of FT-Raman spectroscopy in identifying fillers in some elastomeric products are included, as are peak positions (IR and Raman) used to identify the materials present.

FT-Raman and FT-IR spectroscopy have both been used to investigate and identify[1–5] a large range of components associated with the rubber industry. In this paper we apply the combined and complementary techniques of FT-Raman and FT-IR spectroscopy to the analysis of inorganic fillers and activators and demonstrate where useful information can be gained from one or both of these techniques.

IR spectroscopy has been extensively used to study gum vulcanisates, one of the earliest examples of this being by Corish[6] in 1960 using microtomed sections of vulcanisate. However, it has been much more common to use this technique to identify the polymer, with the filler giving an inconvenient mask to the polymer spectrum, than to use IR to identify the filler.

It has already been demonstrated[7,8] that FT-Raman spectra can be obtained from elastomers reinforced with inorganic fillers and the resulting FT-Raman spectrum used to identify the elastomer. The elastomer identification is possible because the relatively low Raman signals derived from most of the inorganic species used as rubber fillers result in a much lower degree of spectral masking than is the case with IR spectroscopy. However, as the FT-Raman spectra presented here show, where a FT-Raman spectrum is obtainable this can lead to additional information being gained such as crystalline structure or form as well as an identification of the filler.

The FT-Raman experiment is especially useful within a quality control environment, where a quick, reliable answer is required, because of the relatively trivial sampling procedure (the sample is simply placed in the exciting laser path) and the non-destructive nature of the test. This latter characteristic also makes FT-Raman identification useful where individual testing is required to ensure compliance before use (*e.g.* medical items).

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In some instances neither the FT-IR or the Raman can be reliably utilised alone to give a precise identity to the inorganic fillers and/or activators present in a vulcanisate but the combination of both of these techniques provides valuable additional information on which to base an assignment.

It should be noted that carbon black filled samples cannot be studied by FT-Raman spectroscopy and require specialist reflection techniques[9,10] to obtain even the most rudimentary spectra. No carbon black filled samples were examined in this study.

EXPERIMENTAL

All spectra were obtained using a Perkin-Elmer system 2000 combined FT-IR/Raman instrument with gold coated optics. For the FT-Raman experiment a quartz beam splitter and InGaAs detector were used with sample illumination by a diode laser operating at 1.064 μm (near infra-red). In order to obtain FT-IR spectra a KBr beam splitter was used together with a TGS detector.

25 Scans at 4 cm^{-1} resolution were used to collect both FT-IR and FT-Raman spectra, unless otherwise noted, requiring less than 5 min acquisition time per sample.

All samples were standard materials used in the preparation of commercial products with no purification prior to examination.

RESULTS AND DISCUSSION

Zinc Oxide

The most common inorganic additive to be found in any vulcanisate is zinc oxide. The FT-Raman spectrum of zinc oxide is simple with a fairly sharp peak at 441 cm^{-1} and a much less intense peak at 335 cm^{-1}. Unlike many other additives the commercial material examined herein did not show any signs of an organic 'dampener' added to the material. The IR spectrum is also fairly simple with an effective cut off at about 600 cm^{-1}. The FT-Raman and FT-IR spectra of zinc oxide are shown in *Figure 1*.

Titanium Dioxide

Titanium dioxide is usually added to elastomeric goods as a white colour agent and as a brightener. Titanium dioxide occurs in two crystal forms; anatase and rutile. The FT-Raman spectra of these two forms are quite different, the anatase form having peaks at 640, 515 and 397 cm^{-1}, whilst the rutile form has peaks at 610 cm^{-1} and 446 cm^{-1}. The FT-IR spectra of the two materials are much more similar to each other, both having a broad peak between 800 cm^{-1} and 470 cm^{-1} but with the anatase form having a valley in this peak centred around 580 cm^{-1}. *Figure 2* shows the FT-Raman spectra of an NR/SBR latex foam that has titanium dioxide added and overlayed reference spectra of the anatase and rutile forms of titanium oxide. It can be clearly seen from these spectra that the titanium oxide present is in the rutile form.

Other Oxides

Silica (silicon dioxide) has no Raman spectrum, in fact Raman spectra can be collected from samples in glass containers. The IR spectrum is well known[1], with the most intense peak around 1100 cm^{-1}. Variations in the peak positions and the number of peaks can be seen with different forms of silica from amorphous silica to quartz. Amorphous clay,

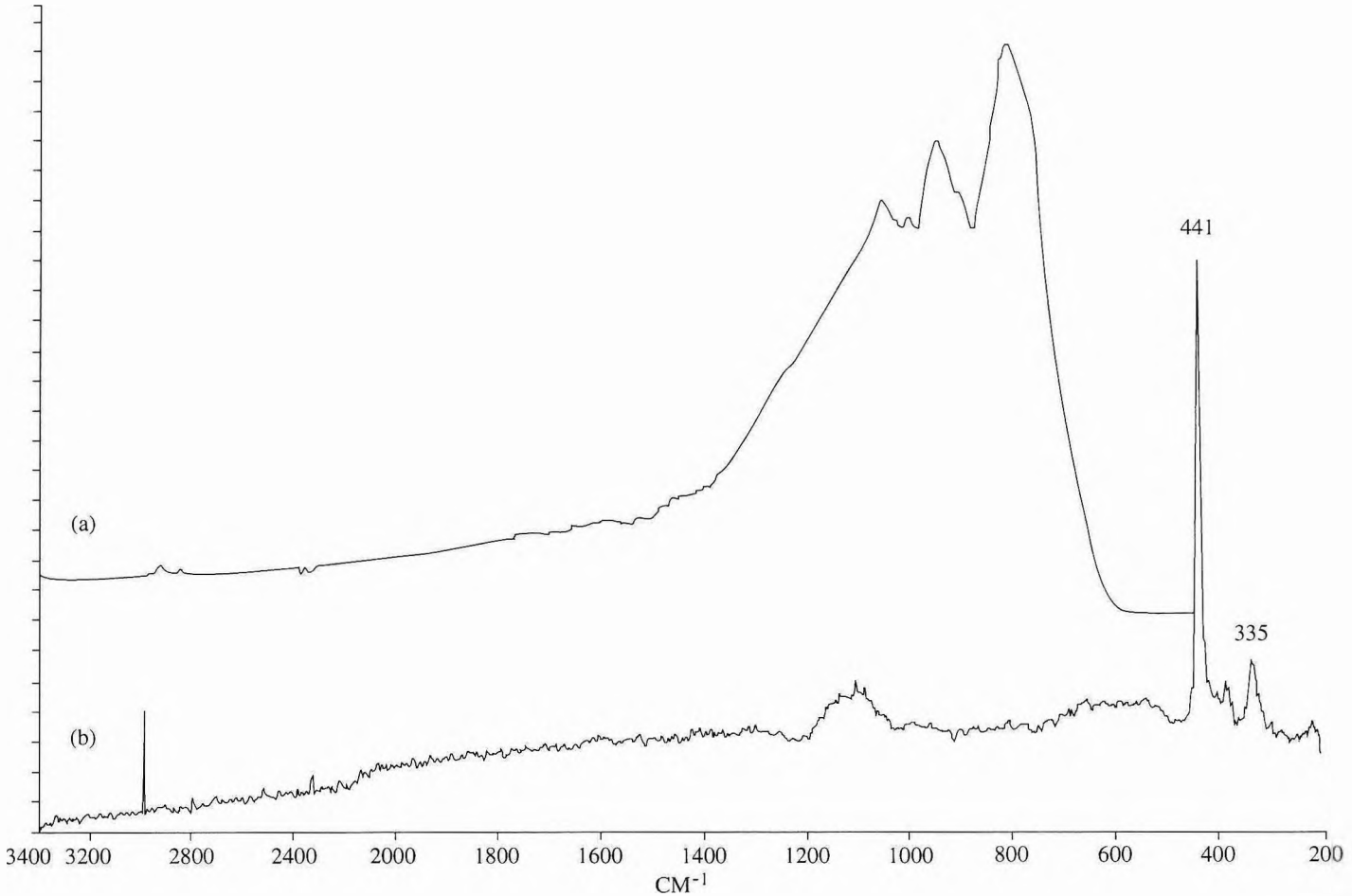

Figure 1. FT-IR (a) and FT-Raman (b) spectra of zinc oxide.

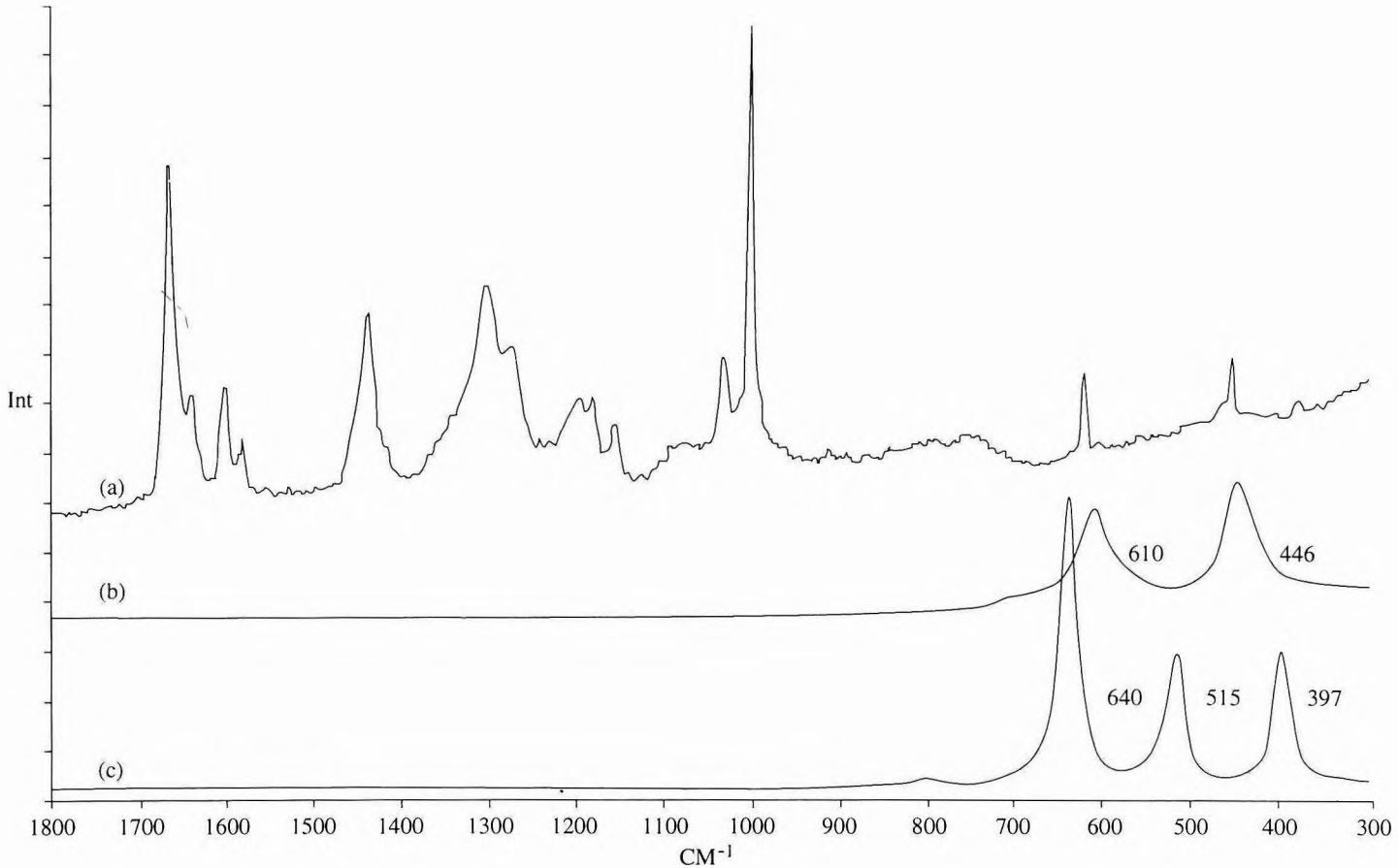

Figure 2. FT-Raman spectra of (a) titanium dioxide filled NR/SBR foam, (b) rutile titanium dioxide and (c) anatase titanium dioxide.

for example, has a very intense, broad peak centred at 1095 cm^{-1} and an additional much less intense broad peak at 800 cm^{-1}, whilst quartz exhibits an intense broad band around 1100 cm^{-1} combined with sharper bands at 795, 775 and 690 cm^{-1}.

Occasionally very broad underlying bands are observed in the FT-Raman spectra (increasing background towards lower wave numbers) of elastomers containing silica, this is indicative of a slightly fluorescent component in the sample mix, other than this there is no indication of the added filler. The FT-IR ATR spectrum is, however, dominated by the silica filler with the 1100 region being particularly affected[7,8].

Magnesium oxide has an IR spectrum very similar to that of zinc oxide, with a broad 'cut-off' starting at approximately 700 cm^{-1}. There are, however, additional weak bands at 1476 cm^{-1} and 1413 cm^{-1} which can be used to differentiate between the two materials. Again, the polar nature of the magnesium oxide molecule precludes a Raman spectrum[11].

Commercial antimony oxide (used as a fire retardant and white colour) has both a Raman and IR spectrum. The IR spectrum is dominated by a broad peak centred at 738 cm^{-1}, with additional unresolved peaks at 580, 537 and 508 cm^{-1}. The FT-Raman spectrum has five fairly sharp peaks at 716 (small), 453, 375, 257 and 192 cm^{-1}, the most intense peaks being at 257 cm^{-1} and 192 cm^{-1}. It is these last two peaks that can be used to determine the presence of antimony oxide in a commercial product. *Figure 3* shows a NR based foam with added antimony oxide. The presence of the fire retardant is clearly demonstrated, with the peaks at 257 cm^{-1} and 192 cm^{-1} showing above that of the polymer.

Carbonates

Calcium, zinc, sodium carbonates and dolomite (calcium magnesium carbonate) are used as additives to elastomer mixes. The most common use for these materials is as a white pigment and as a 'bulking' agent.

The IR spectrum of calcium carbonate is quite characteristic with a very intense broad band centring at 1430 cm^{-1}. In addition there are sharp bands at 1795, 876 and 712 cm^{-1}. The Raman spectrum of calcium carbonate consists of three main, sharp bands at 1089, 716 and 285 cm^{-1}. The identification of calcium carbonate filler in NR is demonstrated in *Figure 4* which shows a FT-Raman spectrum of a vulcanised SMRL with 40 p.h.r. calcium carbonate and a reference FT-Raman spectrum of calcium carbonate. The three bands listed above can be clearly observed in this spectrum making a positive assignment.

Magnesium carbonate gives a Raman spectrum consisting of a single intense line at 1122 cm^{-1} making Raman spectroscopy an unreliable source of identification alone. Supporting evidence for the presence of magnesium carbonate can be gained from the IR spectrum which is characterised by a major absorption at 1482 cm^{-1} and 1420 cm^{-1} and an additional, lower intensity absorptions at 1120, 886, 853, 803, 719 and 593 cm^{-1}.

The IR spectrum of dolomite is, understandably, very similar to that of calcium carbonate but the bands are even broader. The most intense peak is centred at 1440 cm^{-1} –

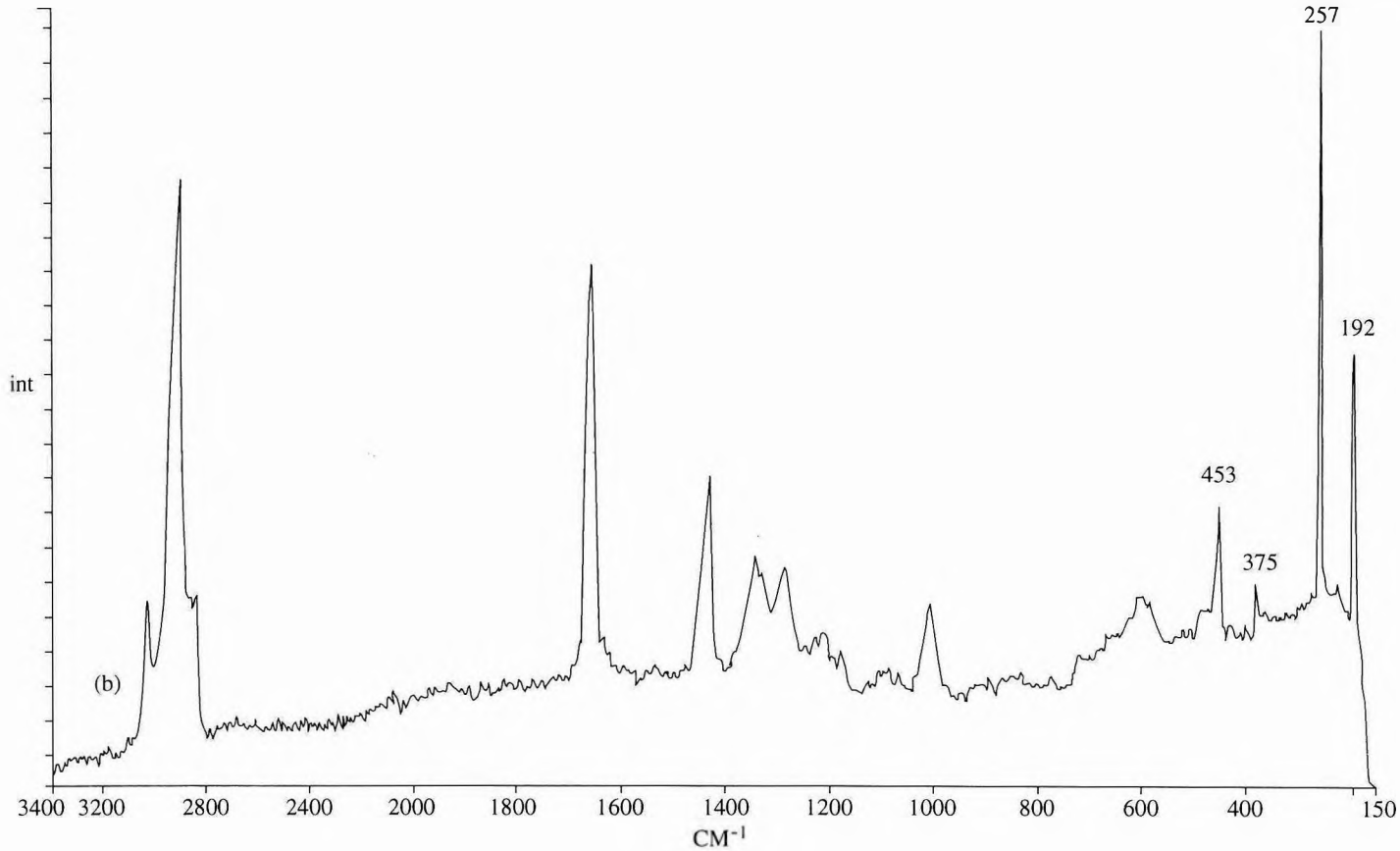

Figure 3. FT-Raman spectrum of an NR foam with antimony oxide fire retardant.

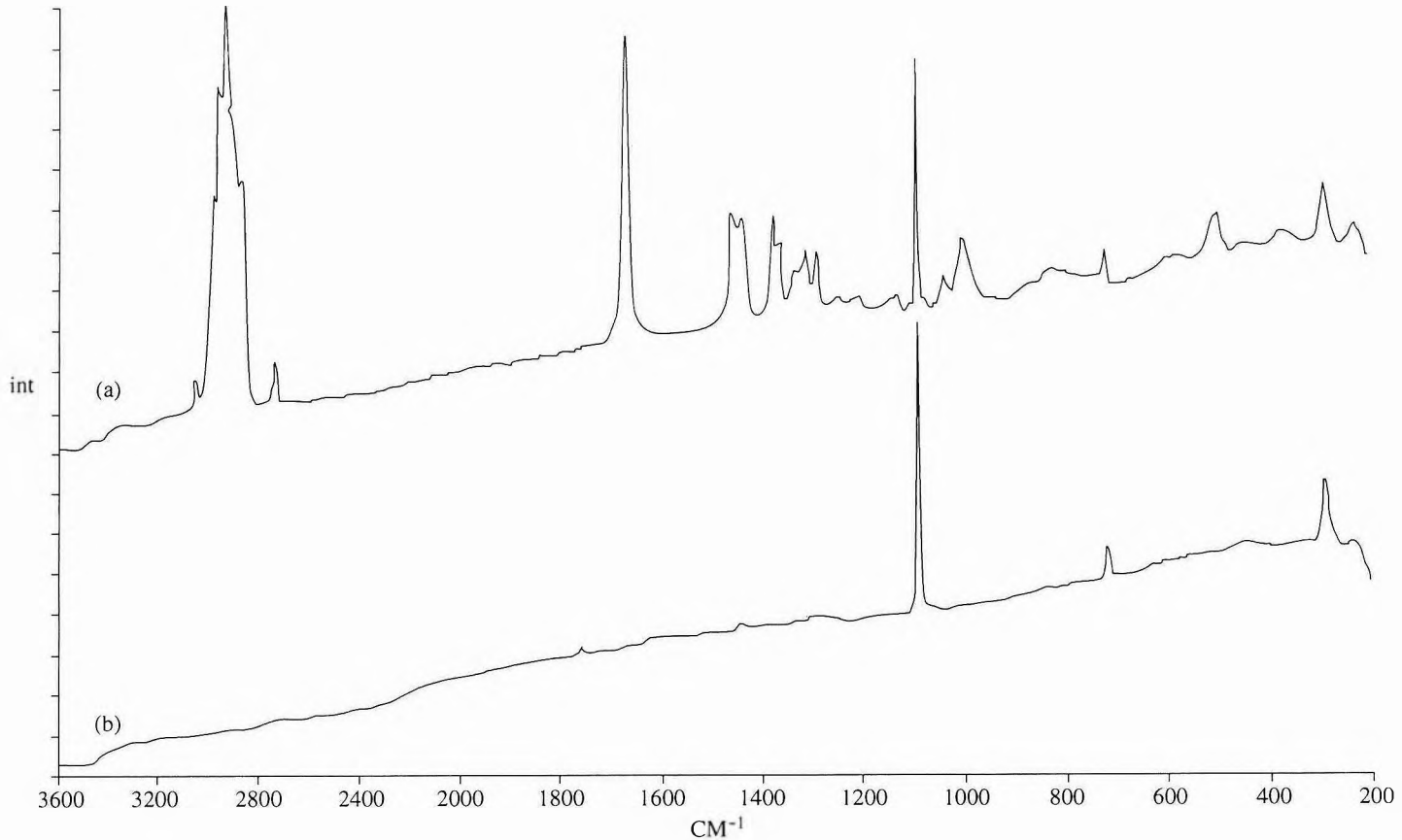

Figure 4. FT-Raman spectrum of (a) an NR vulcanisate filled with 40 p.h.r. calcium carbonate and (b) calcium carbonate

1450 cm^{-1} whilst the other main bands are at 887 cm^{-1} and 734 cm^{-1}. The Raman spectrum is a combination of the peaks found for magnesium and calcium carbonate.

The Raman spectrum of sodium carbonate has peaks at 1081, 1071 and 701 cm^{-1}. There is also a peak near the cut off point of the filters (150 cm^{-1}) but this is an unreliable source for identification. The IR spectrum can be characterised by a broad absorption at 1431 cm^{-1} and two sharp bands at 875 cm^{-1} and 713 cm^{-1}.

Zinc carbonate has a Raman spectrum unlike the other carbonates examined in that the peaks observed are broad. The peaks occur at 1551, 1375, 1065, 985, 738, 712, 391 and 229 cm^{-1} the most intense of these being at 1065 cm^{-1}. It is unlikely that any peak other than the one at 1065 cm^{-1} would be observed in an elastomer mix as the Raman response is very poor for this material. The IR spectrum of zinc carbonate reveals the true nature of the material which is that it is actually a mixture of both the carbonate and the hydroxide and is thus more properly known as basic zinc carbonate or zinc carbonate hydroxide. The IR spectrum has sharp bands in the finger print region at 1046, 951, 834, 738 and 708 cm^{-1}. In addition there are two bands in the area associated with the C=O stretch, the 1384 cm^{-1} band, a position normally associated with inorganic carbonyls and the 1507 cm^{-1} band associated with the presence of the hydroxide. It should also be noted that the OH stretch centred at 3365 cm^{-1} is also greatly increased in intensity beyond that of the other carbonates due to the presence of the hydroxide.

Sulphates

The FT-Raman spectra of calcium and barium sulphate are very similar in composition but with the peaks slightly shifted from each other. The FT-Raman spectrum of calcium sulphate has bands at 1137, 1010 (largest band) 673, 622, 497 and 417 cm^{-1} whilst barium sulphate has bends at 1140, 989 (largest band) 648, 618, 463 and 453 cm^{-1}. The FT-Raman spectra of calcium and barium sulphate are shown in *Figure 5*. The FT-Raman signal from these materials is fairly strong, producing good quality spectra, but practical application of the FT-Raman technique in identifying these materials in elastomers will be difficult for SBRs as the major bands will be very close to that of the styrene peak (1000 cm^{-1}).

The IR spectra of calcium and barium sulphate are also fairly similar, with a broad absorption centred around 1150 cm^{-1} for calcium sulphate and multiple unresolved absorptions with maxima at 1180, 1120 and 1080 cm^{-1} for barium sulphate. The remainder of the spectrum of calcium sulphate depends on whether the anhydrous or hydrated ($2H_2O$) form is present. The hydrated salt has additional bands at 659 cm^{-1} and 600 cm^{-1} as well as the water bands at 3540, 3390 and 1617 cm^{-1} whilst the anhydrous form has sharper bands at 670, 609 and 590 cm^{-1}. Barium sulphate shows bands at 982, 633 and 610 cm^{-1}.

Zinc Sulphide

Zinc sulphide is included in this summary as it is a possible vulcanisation product rather that a normally added ingredient although it is, of course, present in lithopone. The FT-Raman spectrum of zinc sulphide has sharp peaks at 992 cm^{-1} and 352 cm^{-1} and broader, multiple peaks centred at 645 cm^{-1} and 419 cm^{-1}. The IR spectrum of zinc sulphide is limited to a peak at 316 cm^{-1}. This is below the normal

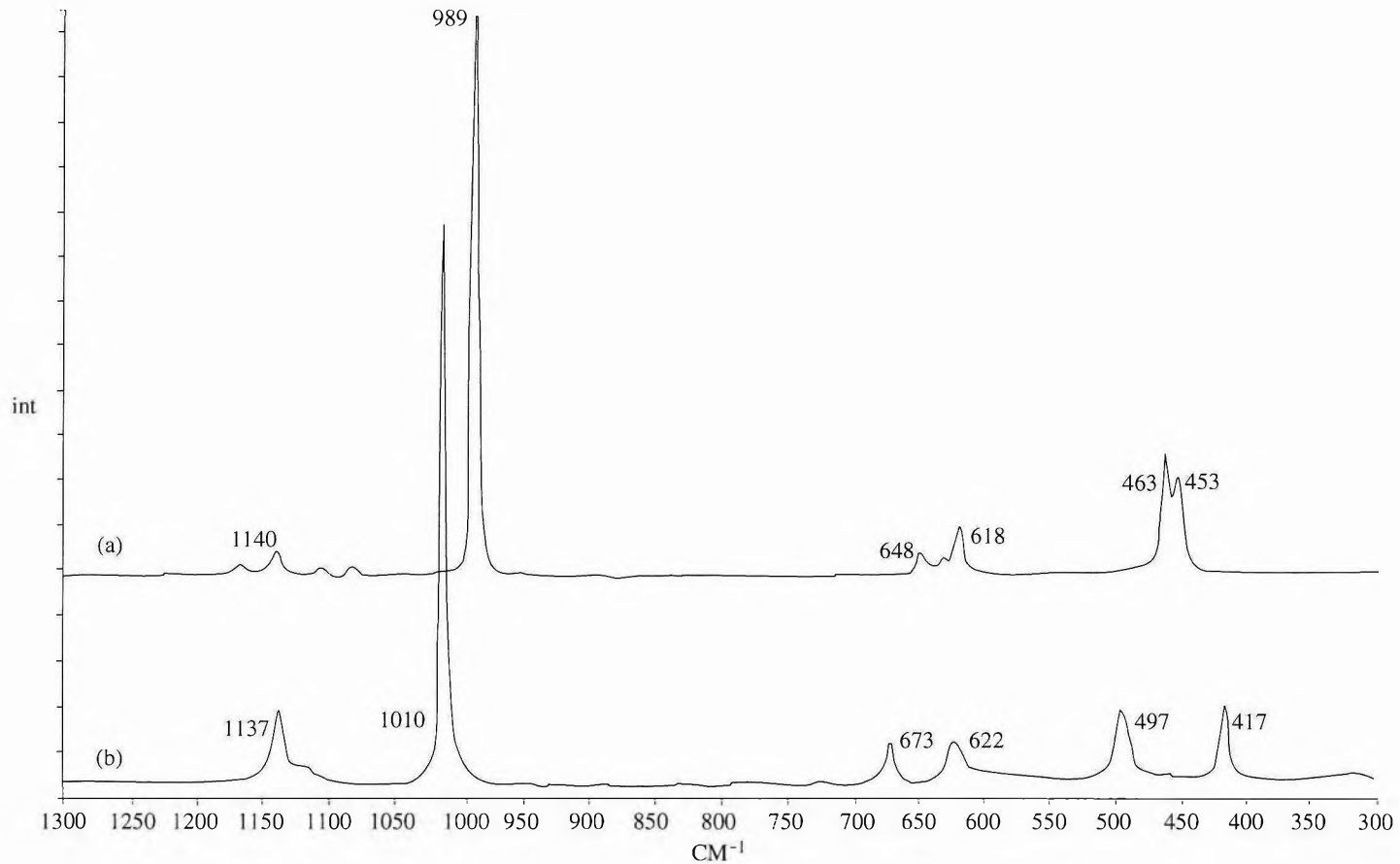

Figure 5. FT-raman spectra of (a) barium sulphate and (b) calcium sulphate.

range scanned and is therefore rarely detected by IR spectroscopy.

CONCLUSIONS

The vibrational spectra of inorganic materials studied in this paper were chosen to represent the more commonly used inorganic fillers, activators and pigments but is not an exhaustive list. The purpose of the paper is to demonstrate the information available from vibrational spectroscopy (both Raman and IR) and to suggest areas in which useful information can be gained.

The three most obvious examples of the use of vibrational spectroscopy in the identification of inorganic elements within a vulcanised elastomer studied herein come from the examination of titanium dioxide, antimony oxide and calcium carbonate. With these three ingredients simply running the FT-Raman spectrum of the whole material would normally be sufficient to demonstrate the presence of these compounds within the vulcanised material. For other components, especially the more polar materials such as silica, the IR spectrum can also be used to identify the inorganic components either by reflectance techniques or by degradation of the sample to leave the inorganic residue.

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Purification of Natural Rubber

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About two long-chain fatty acid ester groups per rubber chain are retained in Hevea *rubber, even after treatment of the rubber with proteolytic enzyme and reprecipitation or acetone extraction. The fatty acid esters and phosphorus compounds were perfectly removed by transesterification of rubber solution with sodium methoxide or saponification with KOH. Almost all the proteins were removed by saponification or deproteinisation, while they remained even after transesterification. The gel content of rubber was reduced to almost zero by transesterification or saponification. Huggins'* k' *constant of the soluble fraction was reduced apparently by these treatments. The branch-points comprising phospholipid esters in* Hevea *rubber were presumed to be decomposed to form linear molecules.*

The basic structure of natural rubber (NR) from *Hevea brasiliensis* has been confirmed to consist of an initiating terminal group, two or three *trans*-isopene units, a long sequence of *cis*-isoprene units and a terminated group aligned in that order[1], although detailed structure of both terminal groups has not been identified. In addition to the isoprene units, small amounts of various abnormal groups such as aldehyde[2], epoxide[3] and lactone[4] have been postulated to be responsible for the occurrence of branching and crosslinking reactions in NR.

Proteins in NR have been regarded as a reactive substance to produce branching by reaction with the abnormal groups[5] and act as an essential component to lead the outstanding properties of NR. However, Ichikawa, *et al.* have shown that the proteins do not participate in the fundamental properties of NR[6]. We have presumed that the long-chain fatty acid groups linked to rubber molecule as phospholipids are one of the major abnormal groups to form branch-points and gels in NR[7]. These findings prompted much attention on the prominent role of proteins, fatty acid groups and phospholipids present in NR. However, the definite structure of the ester linkages in NR even now has not been identified yet.

In this paper, we report about the component and structural changes in NR after different treatments, *i.e.* enzymatic deproteinisation, transesterification with sodium methoxide and saponification with potassium hydroxide. This work provides fundamental information on the whole structure of NR including the branching and gel formation.

EXPERIMENTAL

Fresh field latex (FL-latex) of RRIM 600 clone was preserved in 1% (w/v) sodium dodecyl sulphate for one day before use. The enzymatic deproteinisation was carried out by treatment

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of 10% DRC latex with 0.04% (w/v) *Alcalase 2.0T* and 1% (w/v) *Triton X-100* at 37°C for 24 h followed by centrifugation. The cream rubber was redispersed in 1% (w/v) *Triton X-100* to make 10% DRC and recentrifuged twice. Transesterification was carried out by treatment 1% (w/v) solution of rubber in toluene, with freshly prepared 1 *M* $NaOCH_3$ under N_2 atmosphere in the dark at room temperature for 2.5 h, followed by concentration with a rotary evaporator at 45°C and precipitation in methanol. Saponification was performed by reaction of 1% (w/v) of rubber in hexane/toluene (5:3, v/v) with 1.5 *M* KOH solution in 2-propanol/water (5:1, v/v), in the presence of 0.1% (w/v) methanolic pyrogallol as an antioxidant. The reaction mixture was refluxed at 70°C for 2 h under N_2 atmosphere. The hot saponified mixture was then filtered and washed several times with hot distilled water until the solution became clear-white, then concentrated by evaporation and precipitated in methanol. All the rubbers was further purified by extraction with acetone under N_2 atmosphere for 30 h. The purified rubber was dried *in vacuo* at room temperature and subjected to molecular weight and structural analyses.

All the rubbers were fractionated to several fractions, by solvent fractionation in the usual way[8]. The fractionated rubbers were subjected to analyse the Huggins' *k'* constant, using viscometric measurement[9].

The molecular-weight distribution (MWD) was determined by gel-permeation chromatography (GPC) using two columns in series, packed with styrene-divinylbenzene copolymers having exclusion limits of 2.0 x 10^7 and 5.0 x 10^4. Measurements were made using THF as an eluent, with a flow rate of 0.5 ml/min at 35°C, monitoring with RI and low-angle laser-light scattering (LALLS) detectors. Commercially obtained standard polystyrenes were used for calibration. The purified rubber at concentration of 0.01% (w/v) in THF was filtered through a Millipore LS prefilter and a 0.2 μm before injection.

The gel content was determined by dissolving the purified rubber in dried toluene, which was kept in activated Molecular Sieves 4A, to give a concentration of 0.2% (w/v) and kept in the dark without shaking or stirring for 1 week at room temperature. The solution was centrifuged at 10 000 g for 40 min to separate the gel from sol fraction. The separated gel fraction was dried *in vacuo* and weighed to estimate the gel content.

FTIR measurements were made with a JASCO 5300 FTIR spectrometer. The quantity of the ester groups was determined by FTIR using a calibration curve obtained from a mixture of synthetic *cis*-1,4-polyisoprene and methyl stearate[10]. The nitrogen content was analysed using a Kjeldahl method[11]. The phosphorus content was analysed by digesting rubber with nitric acid according to the method of Moris *et al.*[12] The ^{13}C-NMR spectra were taken with a JEOL λ-500 spectrometer at 50°C in deuterated chloroform.

RESULTS AND DISCUSSION

The gel content in the rubber from FL-latex was reduced from 5% to 3% by deproteinisation and further decreased to about 1% by transesterification or saponification, as shown in *Table 1*. It is generally accepted that the gel fraction of NR is branched molecules which originated from crosslinking reaction due to abnormal groups present in the rubber matrix,

TABLE 1. ANALYSIS OF RUBBERS AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Sample	N content (%, w/w)	Ester content (mmol/kg rubber)	P content (%, w/w)	Gel content (%, w/w)
Control (FL)	0.231	10.4	0.069	5.1
DP-NR	0.016	11.8	0.013	3.2
TE-NR	0.210	~0	~0	0.8
SAP-NR	0.011	~0	~0	1.2

which lead to storage hardening of rubber[5]. The decrease in gel content after these treatments implies that a part of the branch-points was decomposed by deproteinisation and almost all of them were disintegrated by transesterification.

Table 2 shows the change in molecular weight of rubbers after enzymatic deproteinisation, transesterification and saponification, compared with the control rubber. Deproteinisation resulted in an insignificant change in $\overline{M}_n$ and $\overline{M}_w$, while transesterification and saponification reduced the $\overline{M}_w$ and $\overline{M}_n$ to about two-third of the control sample. This indicates that proteins are not concerned with the branch-points observed here, although it was confirmed that proteins are involved in the gel formation[4].

Synthetic *cis*-1,4-polyisoprene was treated in a similar way as the transesterified NR. As shown in *Table 2*, there was practically no change in the molecular weight, suggesting that transesterification is a reaction which decomposes only the ester linkages, which compose the branch-points to form linear molecules.

The Huggins' *k'* constant of the fractionated rubbers from high to low M_w fractions was given in *Table 3*. It is clear that the *k'* values of the TE-NR and SAP-NR were in narrow ranges and smaller than those of the control rubber and DP-NR. It is well-known that the Huggins' *k'* constant of a linear polymer is smaller than that of the branched polymer[13,14]. This indicates that the control rubber and DP-NR contain branched molecules, while the TE-NR and SAP-NR are composed of linear molecules. This result supports the idea that transesterication and saponification breaks down the branch-points which are stable to the enzymatic deproteinisation.

Figure 1 shows FTIR spectra of the rubbers obtained after deproteinisation (DP-NR), transesterification (TE-NR) and saponification (SAP-NR) together with the control (FL-NR). It is clear that the intensity of the infrared band at 3280 cm^{-1}, which is assignable to ν_{NH}, markedly reduced in intensity and shifted to 3316–3320 cm^{-1} as the nitrogen content of the DP-NR and SAP-NR decreased. The characteristic bands of the amide and amine bondings at 1628 cm^{-1} and 1540 cm^{-1} also disappeared in the DP-NR and SAP-NR, while those bands clearly resided in TE-NR and the control rubber. As shown in *Table 1*, the nitrogen content of these rubbers was reduced to about 0.01% by deproteinisation or saponification, but transesterification gave an

TABLE 2. MOLECULAR WEIGHT OF RUBBERS AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Sample	$\overline{M}_w \times 10^{-6}$ (LALLS)	$\overline{M}_w \times 10^{-6}$ (RI)	$\overline{M}_n \times 10^{-5}$ (RI)	$\overline{M}_w/\overline{M}_n$
Control (FL)	2.1	2.4	3.5	6.8
DP-NR[a]	1.9	2.3	3.2	7.1
TE-NR[b]	1.6	1.6	1.9	8.3
SAP-NR[c]	1.7	1.8	2.3	8.0
Cariflex-305[d]	2.2	2.0	5.7	3.3
Cariflex-305-TE[e]	2.0	2.0	5.9	3.4

[a]Deproteinised natural rubber

[b]Transesterified natural rubber

[c]Saponified natural rubber

[d]Synthetic *cis*-1,4-polyisoprene

[e]Transesterified synthetic *cis*-1,4-polyisoprene

TABLE 3. HUGGINS' *K'* CONSTANT OF FRACTIONATED RUBBER AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Rubber fraction	Huggins' *k'* constant			
	Control (FL)	DP-NR	TE-NR	SAP-NR
1	0.65 (6.08)[a]	0.59 (6.11)	0.45 (6.24)	0.4 (6.18)
2	0.55 (4.60)	0.57 (5.36)	0.43 (4.77)	0.43 (5.20)
3	0.57 (3.66)	0.59 (4.19)	0.44 (3.23)	0.40 (4.30)
4	0.50 (2.50)	0.46 (2.19)	0.40 (2.98)	0.42 (2.10)
5	0.45 (1.27)	0.43 (0.68)	0.35 (1.20)	0.36 (0.83)

[a]Values in the parentheses are intrinsic viscosities [η] of each fraction

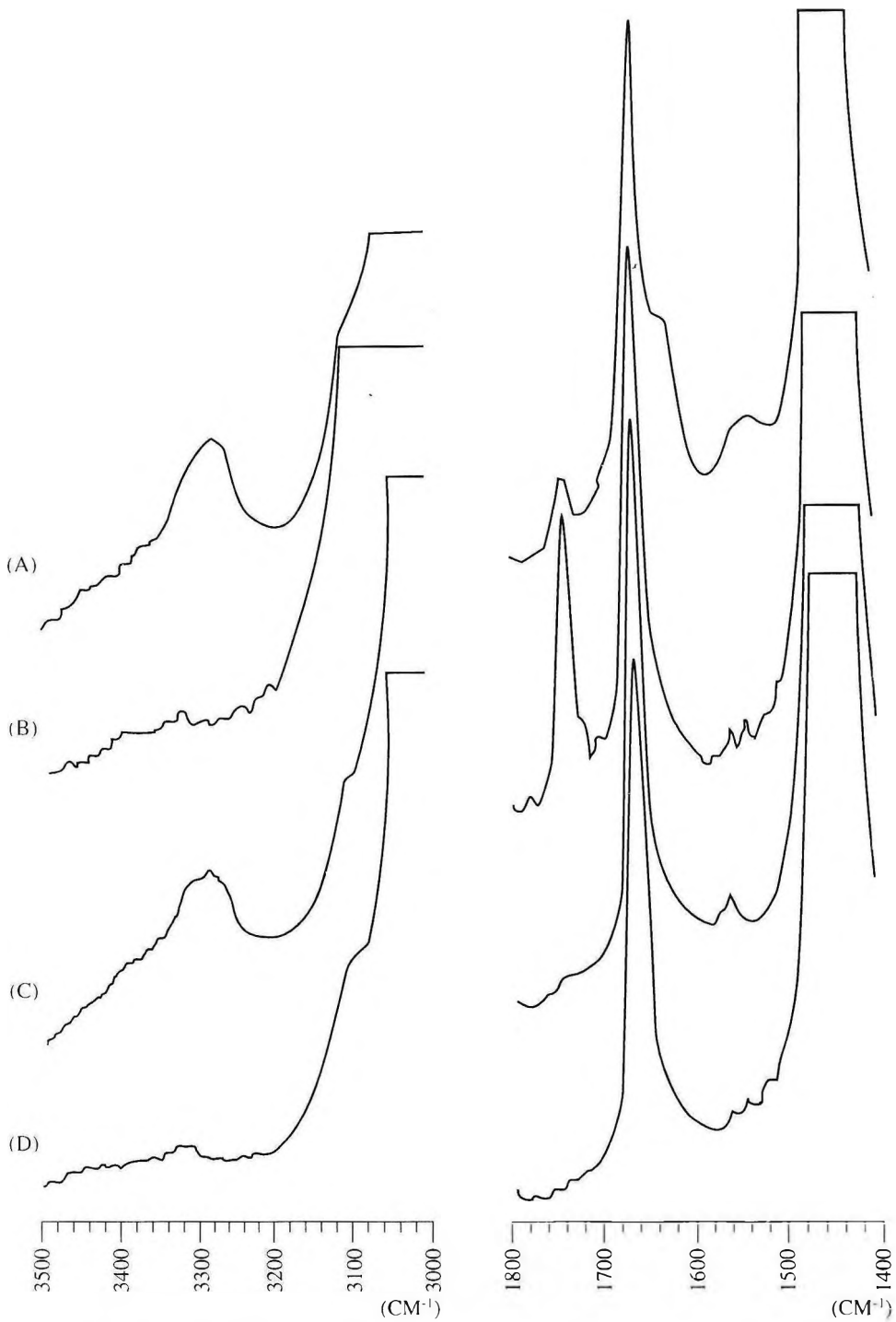

Figure 1. FTIR spectra of the rubbers from (A) control (FL-NR), (B) deproteinised NR (DP-NR), (C) transesterified NR (TE-NR) and (D) saponified NR (SAP-NR).

insignificant change in the nitrogen content. This indicates that the saponification with KOH hydrolyses amide linkages of rubber in hexane/ toluene solution as proteases do in the rubber latex. The residual nitrogen content in the DP-NR and SAP-NR was estimated to be about two to three mol-atom/rubber chain, based on the degree of polymerisation of 5000 for one rubber chain. This quantity of amide groups per rubber chain agrees well with our previous finding that the infrared band at 3316 cm^{-1} to 3320 cm^{-1} corresponds to the N-H band in oligopeptides[15]. Our ^{1}H-NMR study on the structure of NR suggests the presence of a special linkage at the initiating terminal[10]. Although there is no direct evidence to prove the linkage between oligopeptides and this terminal group at present, this finding implies that the amide groups remained in the rubber molecule even after high deproteinisation.

We have shown that the free fatty acid components were removed in the preliminary stage from NR by acetone extraction[7]. However, some fatty acids linked to the rubber chain remained and these could be detected by FTIR and ^{13}C-NMR measurements. Both the control rubber and DP-NR showed a clear infrared band at 1738 cm^{-1} which is a characteristic of ester group in fatty acid esters. This ester bond was confirmed at the long-chain fatty acids linked to the rubber chain and was estimated to be about two molecules per rubber chain. It is clear that this band definitely diminished and the ester content became zero after transesterification or saponification. The removal of the ester groups was also confirmed by ^{13}C-NMR analysis. *Figure 2* (*A*, *B* and *C*) show the ^{13}C-NMR spectra of the DP-NR, SAP-NR and TE-NR rubbers, respectively. The former apparently showed signals at δ 14.0, 29.7 and 34.4, which were corresponding to the terminal methyl ($-\underline{C}H_3$), methylene sequence [$-(\underline{C}H_2)_n-$] and terminal methylene ($-O_2C\underline{C}H_2-$) carbons of a long-chain fatty acid, respectively. These signals completely disappeared after transesterification or sanponfication. The long-chain fatty acids were confirmed to be liberated from rubber chain as methyl esters after transesterification[7]. These facts suggest that the abnormal groups having IR band at 1740 cm^{-1} are fatty acid esters and not as a lactone.

It is clear from *Table 1* that the amount of phosphorus in NR decreases significantly after deproteinisation and became zero after transesterification or saponification. A part of phosphorus constituents such as lipoproteins can be removed in the deproteinisation process. The residual phosphorus content in this stage is calculated about one mol-atom P/rubber chain. This may be regarded as a terminal group derived from the modification of diphosphate group in the termination step of the biosynthetic pathway, which probably is a phospholipid.

It is interesting that both the amounts of phosphorus and fatty acid ester groups decreased to zero after transesterification or saponification. This result supports the assumption that a phospholipid consisting of two molecules of long-chain fatty acids which probably originate the phosphorus and long-chain fatty acid components in NR. The reduction of gel content, molecular weight and Huggins' *k'* constant of rubbers after transesterification or saponification also implies that the phospholipid should be a principle component to produce the branch-points in NR. The detailed structure of the branch-points will be discussed in a subsequent paper.

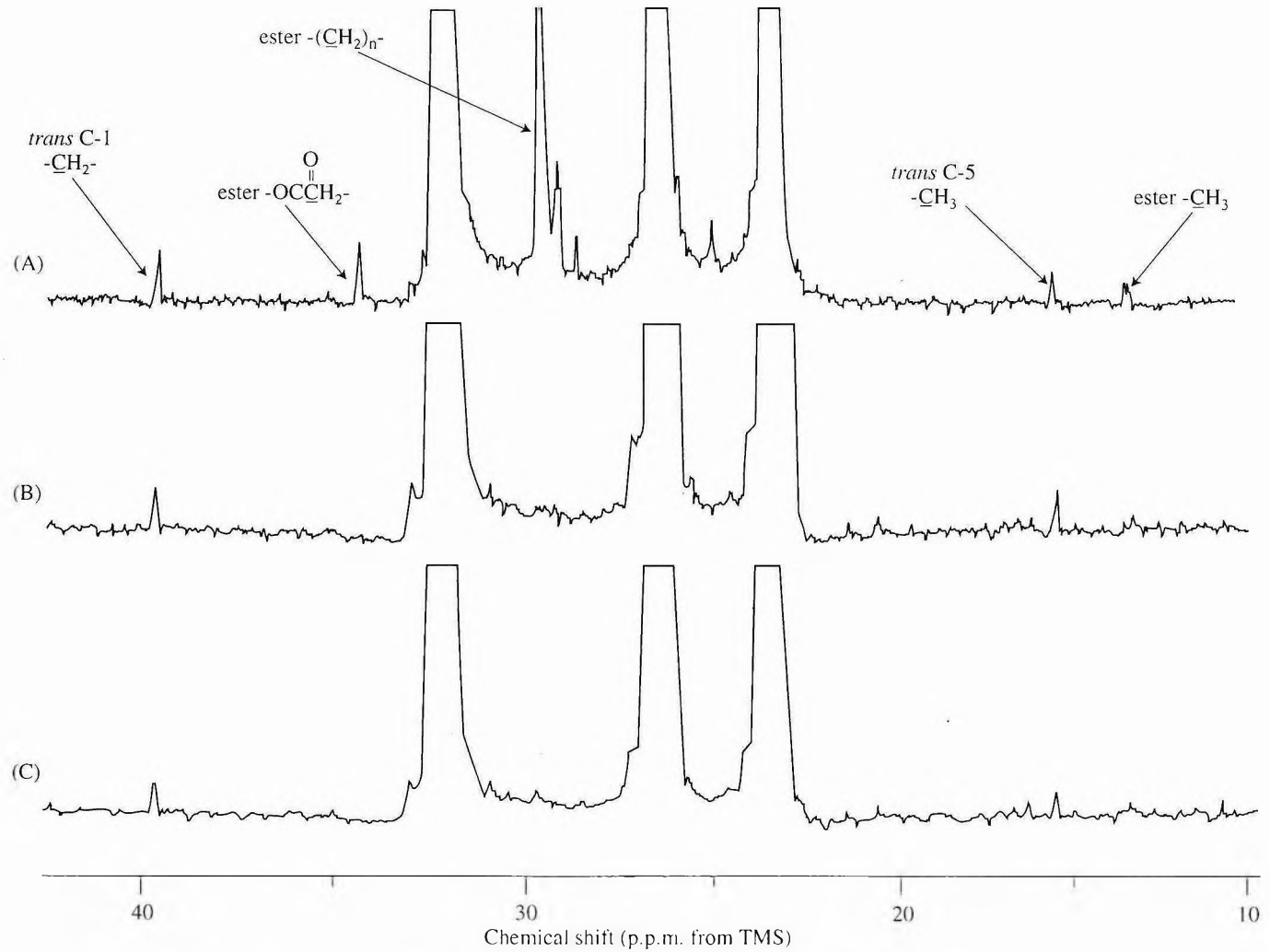

Figure 2. ^{13}C-NMR spectra of (A) deproteinised NR (DP-NR), (B) transesterified NR (TE-NR) and (C) saponified NR (SAP-NR).

CONCLUSION

Enzymatic deproteinisation breaks down the protein linkages selectively and remained as oligopeptide groups. The phospholipid ester in NR was severed by transesterification, resulting in the linear rubbers as the products. Saponification can remove both of the protein and phospholipid linkages to form the linear rubber molecules like those obtained from deproteinisation followed by transesterification. Transesterification and saponification gave the reduction in gel content, k' and molecular weight, showing that the branch-points might be composed of phospholipid.

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Correlation between Total Extractable Proteins and Allergen Levels of Natural Rubber Latex Gloves

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Certain proteins or peptides eluting from natural rubber (NR) latex products can cause immediate hypersensitivity reactions (Type I allergy) in subjects sensitised to them. The amount of total extractable proteins in manufactured latex products is believed to reflect reasonably well their corresponding allergenic protein level, but only a few studies have been published to substantiate this. The aim of the present study is to compare a widely used total protein measurement assay, namely, the RRIM modified Lowry test (EP_{RRIM}), to latex allergen analysis, carried out by specific IgE-ELISA-inhibition tests. A series of 46 widely marketed medical NR latex gloves was investigated. Their EP_{RRIM} values ranged from < 20 μg/g to 1290 μg/g, and their allergen content varied from < 1 AU/ml to 570 AU/ml. In the measurement of allergen contents, the reference allergen mixture was prepared from serum proteins of fresh Hevea *latex, and IgE antibodies were sourced from both adults and* spina bifida *children sensitive to latex. Results showed that the allergen levels were very well correlated with the total extractable protein contents (coefficient r = 0.89, P<0.001, n = 46). With the exception of a few, gloves with high total extractable proteins were generally found to have high allergen contents, and* vice versa. *Gloves with EP_{RRIM} levels of 0.1 mg/g or 100 μg/g and below always had very low allergen contents (< 9 AU/ml).*

These findings are consistent with those shown by the in-vivo *skin-prick test reported earlier. More importantly, they confirm the very low allergen levels observed at EP_{RRIM} levels of about 100 μg/g and lower. Such information provides useful guideline for the manufacturing of reduced risk NR latex gloves.*

Type I allergy affecting certain individuals through the use of some NR latex products, has caused great concern among the latex product manufacturers. Emphasis has since been placed on the production of latex devices with better biocompatibility. Many attempts have therefore been made to reduce their total extractable proteins shown to be implicated in

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the allergy reaction[1,2]. A number of effective methods for such reduction have subsequently been developed in Malaysia. These include the use of low protein latices[3–5], suitable leaching protocols during processing[4,6], enzyme treatment[7], chlorination of the finished products[8] and polymer coating. When applied under suitable conditions, the extractable protein fraction can be effectively reduced to a very low level.

It is noteworthy that the residual extractable fraction of latex products may consist of both the allergenic proteins (*i.e.* capable of binding to IgE antibodies) and non-allergenic proteins, the proportion of which may vary from product to product. It is, therefore, of great importance that reduced levels of extractable proteins reflect the reduced allergen level of the final products. The present work was thus undertaken to study the correlation between total extractable proteins and allergen contents of latex gloves. Results were also compared with those reported earlier[9] on the relationship between allergic response elicited in latex hypersensitive subjects and total extractable proteins of latex gloves.

METHODS

Extractable Protein Content — RRIM Modified Lowry Method [*Malaysian Standard Test Method MS 1392 : 1996 (P)*][10]

Protein extraction. Cut pieces, of 7 cm x 7 cm each from palm area of glove sample were extracted in 0.01 *M* phosphate buffered saline at pH 7.4 (1 g/5 ml) at 23°C for 3 h with agitations at 30 min intervals. The clear extract was obtained after removing any insoluble matter that might be present by centrifugation.

Protein precipitation. Proteins in the extracts were precipitated using trichloroacetic acid (4.4%, w/v) and phosphotungstic acid (0.2%, w/v). The precipitated proteins were then sedimented by centrifugation at 10 000 x g for 30 min. The resulting protein pellet was redissolved in minimum volume of 0.2 *M* sodium hydroxide (1 ml - 6 ml) after removal of the supernatant containing interfering substances.

Modified Lowry microassay. 0.8 ml aliquot of each redissolved protein solution was treated with 0.3 ml of a reagent containing 6% sodium carbonate and 1.5% copper sulphate in 3% sodium citrate (carbonate: sulphate = 10 : 0.2). After allowing the mixture to stand for 10 minutes, 0.1 ml of 72% 2 *N* Folin reagent was added. Colour was allowed to develop at room temperature for 30 min, and its absorbance at 750 nm was recorded. If precipitation occurred at this stage, further centrifugation was carried out to give clear solution for the colorimetric measurements. Results were read against a standard Bovine Serum Albumin (BSA) calibration curve and converted to μg/g or mg/g of gloves, taking into consideration the weight of sample extracted and the volume used in each case.

Allergen Content—ELISA-inhibition Test[11]

Latex serum proteins containing the allergens. Frozen (–70°C) non-ammoniated *Hevea brasiliensis* latex harvested freshly from the trees under chilled conditions, was thawed and centrifuged to give a clear serum containing latex allergens (NRL serum : protein concentration 10 mg/ml, as measured by Lowry assay). It was diluted to a protein concentration of 20 μg/ml in 50 mM carbonate buffer at pH 9.6 and applied onto polystyrene microtitre plate (100 μl per well; Nunc, Denmark),

incubated at room temperature for 3 h. The wells were emptied and post-coated with 1% human serum albumin in 50 mM carbonate buffer.

Inhibition and immunoassay. The IgE serum pool for the inhibition reaction consisted of carefully characterised sera from both latex allergic adults (n=3) and latex allergic children with *spina bifida* (n=3). Optimally diluted IgE serum pool was mixed with equal volume of each of the serially diluted glove extract (both in phosphate buffered saline-Tween-human serum albumin) and the mixture incubated for 1 h at room temperature. The inhibited mixtures were then introduced into the coated microtitre plate and incubated for a further 2 h at room temperature. After appropriate washes, the bound IgE was detected by biotinylated goat anti-human IgE (Vector) and streptavidin-conjugated alkaline phosphatase (Bio-Rad). Intensity of colour formed upon reaction with the substrate development solution (Sigma) was read at 405 nm.

A standard curve for the inhibition reaction was obtained, based on serial 10-fold dilutions of the NRL serum containing 10 mg/ml of proteins to which 100 000 arbitrary allergen units (AU/ml) were assigned. OD reading of each test sample was converted to these units from the standard curve.

RESULTS

In the present study, relationship between total extractable proteins (EP_{RRIM}), as determined by the RRIM modified Lowry test, and allergen content/activity, as assessed by the ELISA-inhibition test, was investigated. The reference allergen mixture consisted of serum proteins from fresh *Hevea* latex, while IgE antibodies were sourced from both adults and *spina bifida* children who showed sensitivity to latex. Allergen measurements so generated using these reference mixtures have been shown to be highly correlated to the allergic response by the skin prick test (correlation coefficient $r = 0.94$, $P < 0.001$, $n = 20$)[12], indicating the reliability of the test for allergen quantitation. Accordingly, allergen content of < 10 AU/ml is low, 10 –100 AU/ml is moderate, and > 100 AU/ml is high.

A total of 46 commercially available brands of medical latex gloves of which 11 were powder-free, were examined. Their extractable protein (EP_{RRIM}) content varied from as low as less than 20 µg/g (or < 0.02 mg/g) to as high as 1290 µg/g (or 1.290 mg/g), and their allergen content ranged from < 1 AU/ml to 570 AU/ml (*Table 1*). Median values of the gloves samples were 485 µg/g and 117 AU/ml, respectively. Generally, gloves with high protein values had high allergen contents and *vice versa*. However, there were some exceptions, such as in the case of samples No.16, 20 and 22 showing higher allergen contents than the trend depicted. Similarly, samples No.11, 21 and 26 indicated lower allergen levels than expected. These could well be attributed to variability in protein composition of the residual fraction in the samples concerned, due to marked differences in the processing conditions employed. It is most apparent that at EP_{RRIM} levels of about 100 µg/g or 0.1 mg/g and less, the corresponding allergen contents are consistently and remarkably low at < 9 AU/ml (*Figure 1*). This latter group of gloves consisted of all the 11 powder free gloves examined, in addition to 3 powdered ones. Statistical analysis revealed that the two parameters are well correlated, with the coefficient of correlation $r = 0.89$, $P < 0.001$ (*Figure 2*).

TABLE 1. TOTAL EXTRACTABLE PROTEIN AND ALLERGEN LEVELS IN 46 BRANDS OF NR LATEX MEDICAL GLOVES

Glove brand (Sample No.)	Total extractable protein content		Allergen level
	µg/ml of extract	µg/g of glove	AU/ml extract
1	258	1290	510
2	243	1215	570
3	229	1145	410
4	228	1140	323
5	221	1105	378
6	219	1095	299
7	211	1055	350
8	201	1005	378
9	197	985	239
10	184	920	233
11	183	915	125
12	178	890	307
13	175	875	212
14	167	835	350
15	148	740	264
16	144	720	510
17	142	710	257
18	130	650	188
19	129	645	239
20	121	605	388
21	109	545	105
22	106	530	496
23	102	510	212
24	91	457	145
25	89	445	212
26	86	430	81
27	77	385	197
28	69	345	135
29	65	325	128
30	51	255	128

TABLE 1. TOTAL EXTRACTABLE PROTEIN AND ALLERGEN LEVELS IN 46 BRANDS OF NR LATEX MEDICAL GLOVES (CONT.)

Glove brand (Sample No.)	Total extractable protein content μg/ml of extract	μg/g of glove	Allergen level AU/ml extract
31	48	240	115
32*	21	105	4
33	16	80	3
34	16	80	8
35	13	65	5
36	12	60	2
37*	12	60	8
38*	9	45	5
39*	9	45	4
40*	8	40	4
41*	8	40	5
42*	6	30	3
43*	5	25	2
44*	4	20	< 1
45*	< 4	< 20	3
46*	< 4	< 20	3

*Powder free gloves

Extraction ratio: 5 ml of water per gram of gloves

Allergen levels[12]: < 10 AU/ml – Low; 10 to 100 AU/ml – Moderate; > 100 AU/ml – High

DISCUSSION

Allergenicity

Allergenicity or allergic potential of latex products with reference to latex protein allergy has recently become a parameter of importance in the manufacturing of safer latex articles of low allergen quality, particularly the medical devices. There are, however, no standardised methods for such measurement to-date. Several tests, most of them competitive immunoassays, are commonly employed by various laboratories in the West. These include mainly the radioallergosorbent test with inhibition (RAST-inhibition)[13,14] and the enzyme-linked immunosorbent test with inhibition (ELISA-inhibition)[12]. In these *in-vitro* tests, latex allergens are quantified by allowing the soluble latex allergens in the sample extract to compete with a reference allergen mixture adsorbed on a solid phase, for the binding sites of a pool of latex-specific human antibodies. The amount of the IgE latex-specific antibodies bound to

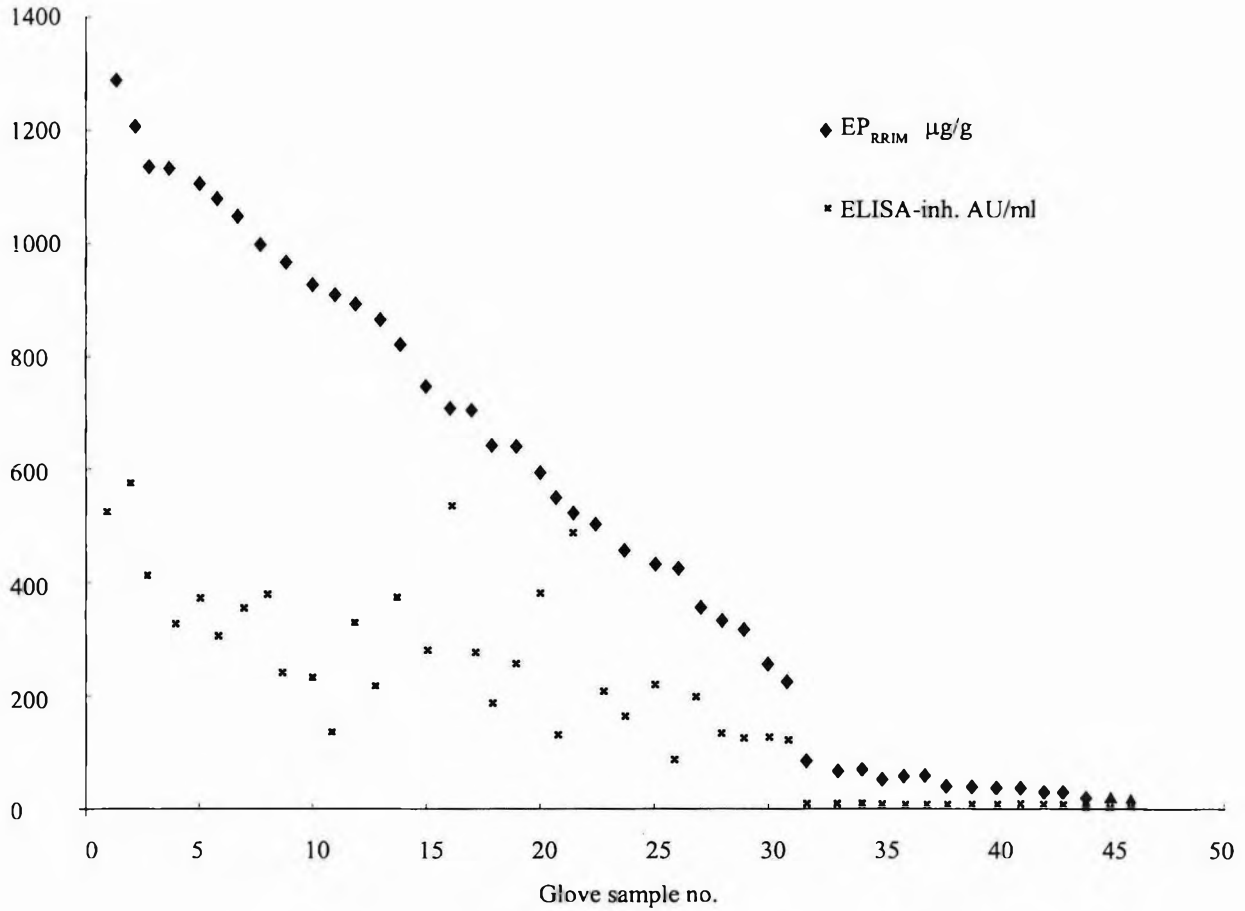

Figure 1. Total extractable proteins (EP_{RRIM}), as determined by RRIM modified Lowry test, and the corresponding allergen content, as assessed by the ELISA-inhibition test, for 46 brands of medical latex gloves.

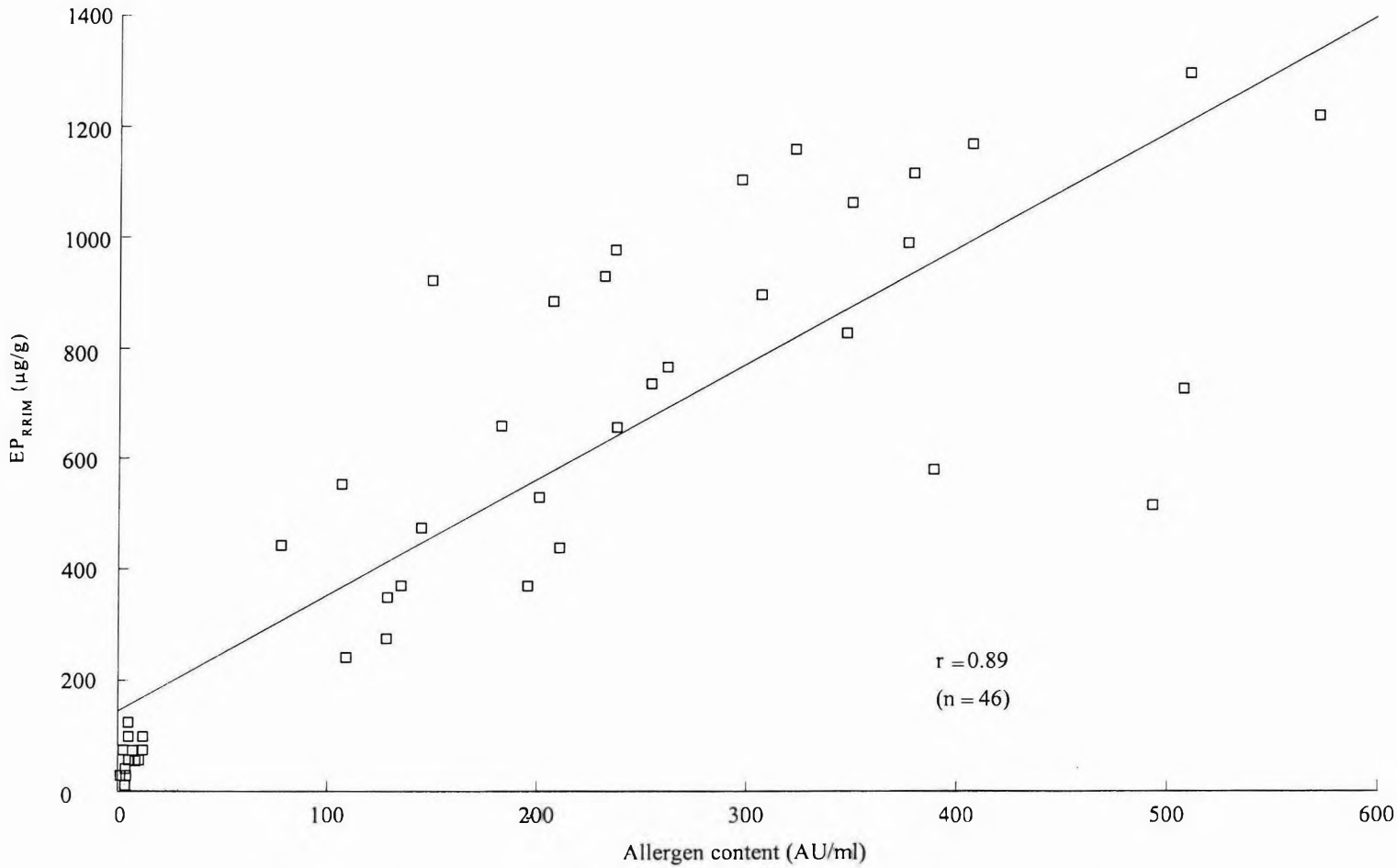

Figure 2. Correlation between total extractable protein contents as determined by the RRIM modified Lowry test, and their corresponding allergen levels as assessed by the IgE ELISA-inhibition assay, for 46 commercially available medical NR latex gloves.

the solid phase is determined, and is inversely proportional to the quantity of latex allergens in the test sample. Resulting measurements are expressed as allergen content/activity in AU/ml. For effective measurements, the reference latex protein standard should contain all relevant allergens, and the reference serum pool should comprise a complement of the corresponding latex specific IgE antibodies. Unfortunately, such standard allergen mixture and IgE serum pool have yet to be developed. In view of this, absolute values of these measurements may vary when either or both the reference allergen mixture and the IgE antibodies used differ. Hence, there is a need to correlate results obtained between laboratories when latex allergens and IgE antibodies from different sources are used.

On the other hand, allergenicity can be specifically evaluated by the *in-vivo* clinical skin prick test, by assessing the allergic response elicited by the protein extract in latex hypersensitive subjects[9,12,14,15]. This test is both sensitive and specific. But the availability of latex sensitive persons is essential. This requirement is not always easily met, especially in latex product manufacturing countries such as Malaysia where prevalence even among the high risk groups has been shown to be very low[16].

Total Extractable Proteins

By far, the colorimetric measurement of total extractable proteins in latex devices offers a relatively simple procedure, involving standard chemicals which can be obtained easily. It has therefore, been adopted for routine monitoring of protein reduction during manufacturing. The modified Lowry microassay protocol which is often used consists of three parts, namely, protein extraction, protein precipitation and the colorimetric microassay. Only the commercially available chemicals are used in this test, and the testing time is (excluding protein extraction time) 2 h – 3 h, as compared to 1–2 days for the immunoassays. Results are expressed in mg/g or μg/g of test sample, with reference to a standard protein. One drawback of this test concerns the fact that it measures all the extractable proteins some of which may not be allergenic. Therefore, for the values generated to be meaningful, they should be related to the allergenicity or the allergen levels.

Relationship

There had been some speculations that total extractable proteins were not correlated to their allergenicity or allergen contents. However, this has been shown to be not so. A good correlation between the two parameters has in fact been shown by Yip *et al.*[9], who assessed the skin prick test allergic response elicited by 39 glove extracts with EP_{RRIM} varying from < 20 μg/g to >1000 μg/g, in a total of 59 latex hypersensitive subjects. The coefficient of correlation, 'r' was 0.83 at $P< 0.001$. This is consistent with the report by Yunginger *et al.*[13] who indicated a significant correlation between extractable protein content of 71 latex gloves, as determined by a modified ninhydrin method, and their allergen levels as assessed by IgE-RAST inhibition immunoassay. Further substantiation is now obtained in the present study which demonstrated a highly significant correlation between the total extractable proteins and the allergen levels of latex gloves, as evaluated by the IgE-ELISA-inhibition immunoassay.

It has been shown that soluble proteins migrate towards the surface of a latex film[17,18]

during the manufacturing process of latex-dipped products. The degree of removal of these proteins from the surface is very much influenced by the processing conditions employed, some being more effective than others[19]. In view of the fact that the residual extractable proteins may not all be allergenic, the doubt often arises as to whether there is any preference in the removal between the non-allergenic and allergenic proteins or among the various allergenic proteins from this fraction. The relationships observed between total extractable proteins and allergen levels and allergenicity[9] suggest that reduction affects both types of proteins, and the existence of some preferences has also been implied.

It is also of interest to note that when reduction reaches a very low EP level of about 100 μg/g and less, evidence strongly indicated that the amount of allergens present, if any, is often too little to facilitate substantial binding with latex-specific IgE or elicit any allergic response in latex hypersensitive persons, as demonstrated by the tests conducted. This is regardless of whether the gloves are powdered or powder free. Hence latex products with such low extractable protein levels can be considered to be of low risk to users. The availability and use of such latex products are expected to reduce or even to prevent further sensitisation. However, it should be stressed that there is a small number of highly atopic people who are sensitive to a great number of allergens. For these subjects, even minute amounts of allergens can elicit hypersensitivity reactions, implying avoidance of all the relevant allergens should be recommended.

It may be mentioned that since absolute values of total extractable proteins generated by different colorimetric methods are not fully comparable[20], results of the present study are relevant only to those determined by the RRIM modified Lowry test. This has consequently rendered the test a convenient and a useful one for monitoring purposes in the manufacture of low protein latex products.

CONCLUSION

Total extractable protein contents of latex gloves (EP_{RRIM}), as determined by the RRIM modified Lowry test, have been found to correlate well with their allergen contents as assessed by the IgE-ELISA-inhibition test. Gloves with high total extractable protein contents are generally associated with high allergen contents, while those with low total extractable proteins tend to have low allergen contents. Protein levels with minimal allergen content/activity have been identified to be about 100 μg/g and lower. These findings are highly consistent with those reported for EP_{RRIM} values and allergic responses by the skin prick test. Such information provides very useful guidelines not only for the manufacture of the more bio-friendly low protein latex products, but also for users in their selection of gloves.

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