

Visualisation of the Network Structure in Some Environmentally Degraded Natural Rubber Gloves

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Transmission electron microscopy (TEM) techniques involving swollen rubber samples in a polymerised matrix were used to characterise the network structure of natural rubber glove pieces, which had been degraded in soils and in compost. TEM analysis of changes in the network structure of the rubber latex particles was used together with Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopy to study various stages of degradation. The extent of degradation was visualised as the breakdown of the rubber latex particles into a loose network of chain structures, with the accompaniment of considerable reduction in the number of cis double bonds and the emergence of oxygenated structures. These observations however, could not be related to the actual physical loss in mass of degraded materials, indicating that the degradation process did not occur uniformly on any given piece of material left to decompose.

Key words FTIR-ATR, NR, gloves, environment, transmission electron microscopy, network structure, latex particles, degradation

The natural rubber (NR) glove is one of the major rubber products derived from a coagulant dipping process. As a product of a natural resource, the biodegradability of NR gloves had been described in a number of reports^{1–6}. Adequate work has been done in establishing the metabolic intermediates of NR biodegradation and it is concluded that the primary mechanism in NR biodegradation involves the cleavage of double bonds that affects the overall structure of a rubber network^{1,4,7}.

Cook *et al*⁸ described a technique involving the analysis of stained embedded swollen samples in a polystyrene matrix using

transmission electron microscopy (TEM). It was shown that by employing this technique, the microstructure of the latex film could be revealed. Consequently, the crosslink density in different types of pre-vulcanised latex films could be compared. The extent of degradation in soil burial tests is usually indicated by the loss in mass and tensile properties, and a decline in solvent swelling index. In the present work, the changes in the microstructure of gloves degraded in sandy and clayey soils, and in a composting environment were observed *via* network visualisation and Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopy.

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MATERIAL AND METHODS

Gloves and Treatments

The glove pieces used were from a soil burial experiment described previously⁵ and from a composting experiment.

In the soil tests, weighed pieces of powdered examination gloves were buried in unamended heavy-clay Munchong series (Typic Hapludox) and a sandy Holyrood series soil (Typic Quartzipsamment) in large plastic trays and incubated at 40% field capacity. Gloves samples retrieved from soils were washed in running water and dried (60°C, 24 h) prior to weighing. The pieces retained 64% and 63% of their initial weights at 12 weeks, respectively, for samples from the clayey and sandy soils, and 21% and 57% at 24 weeks.

In the composting experiment, the NR glove pieces were placed in nylon net (38 µm pore size) mesh bags within a compost mix developed from dried grass clippings, cattle manure and soil (6:3:1, v/v). The compost pile was maintained at 40% moisture within 43 × 51 (D × H) cm² PVC compost bins, and the pile turned over manually at seven-day intervals. Pile temperatures were monitored daily, and the moisture adjusted weekly to prevent inhibition of microbial activity. The composting process was operated for 24 weeks. The pieces from the composting pile had 55% and 27% of their initial weights remaining at 12 weeks and 24 weeks.

Preparation of Samples for Network Visualisation

The glove samples were continuously extracted with cold acetone (Analar) in a Soxhlet apparatus for 24 h at room temperature

and left to stand for 2 h to dry after the extraction. Subsequent drying was carried out overnight in vacuum at room temperature.

The extracted glove samples were then swollen to equilibrium in 0.45 % w/w of benzoyl peroxide in styrene (99%, Aldrich) and 0.9% di-n-butyl phthalate. Prior to use, the styrene was washed with sodium hydroxide (1 M) solution and water to remove the inhibitor that is present in commercially available styrene, and then dried over anhydrous calcium chloride. Diphenylpicryl hydrazyl (9.1 mg/L styrene) was then added to provide effective antioxidant protection and to confer a period of peroxide inhibition prior to styrene polymerisation. Portions of the swollen glove samples in excess styrene were further heated in a gelatine capsule at 68°C for 48 h or until the styrene had fully polymerised.

Transmission Electron Microscopy

Ultra-thin sections (≈ 100–150 nm) were cut from the swollen embedded samples at –110°C with an RMC MT-7000 ultramicrotome using 45° freshly cleaved glass knives set at a shallow clearance angle. The sections obtained were placed on grids that had previously been dipped in ethanol in order to aid sample collection. The sections were stained with osmium tetroxide vapour for one hour. The stained sections were then examined using a Philips EM 300 Transmission Electron Microscope operating at 100 kV with a magnification of 36 000 times.

FT-IR Spectroscopy

The spectra of the glove surfaces were obtained using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer with ATR accessory

(Specac MK II Golden Gate Ge-ATR). A total of 50 scans were accumulated with a resolution of 4 cm^{-1} .

RESULTS

Network-visualisation

Osmium tetroxide preferentially stains carbon-carbon double bonds; the dark phase in the micrographs is rubber and the light phase is polystyrene. In an undegraded glove, swollen individual rubber latex particles appeared as dark areas of threadlike mesh with distinct boundaries surrounded by white areas of polystyrene matrix (*Figure 1*). By contrast, in

the gloves, which had been degraded in soil, nearly all of this latex particle structure was destroyed (*Figure 2*). Also, in the undegraded glove the particles were linked fairly closely together by a loose network of rubber threads, but in the degraded gloves large areas of polystyrene infiltrated the weaknesses in the overall structure of the rubber network.

In the case of the gloves, which had undergone composting, more of the latex particle structures were retained although several regions exhibited a low network density where large amounts of polystyrene were visible (*Figure 3*). The 24-week-old glove sample displayed more areas of a low network density than the 12-week-old sample.

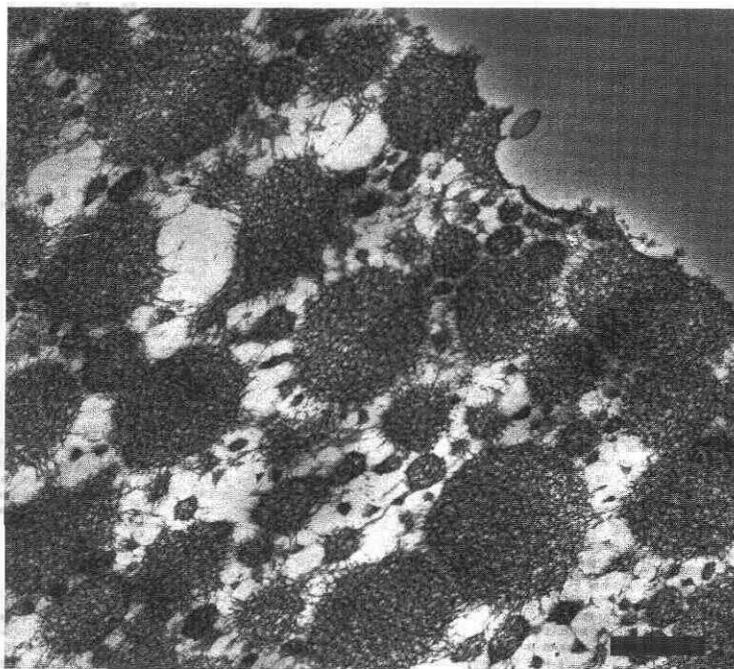
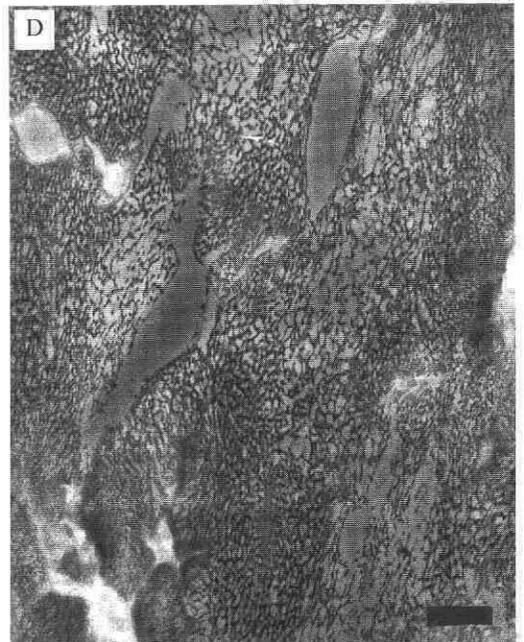
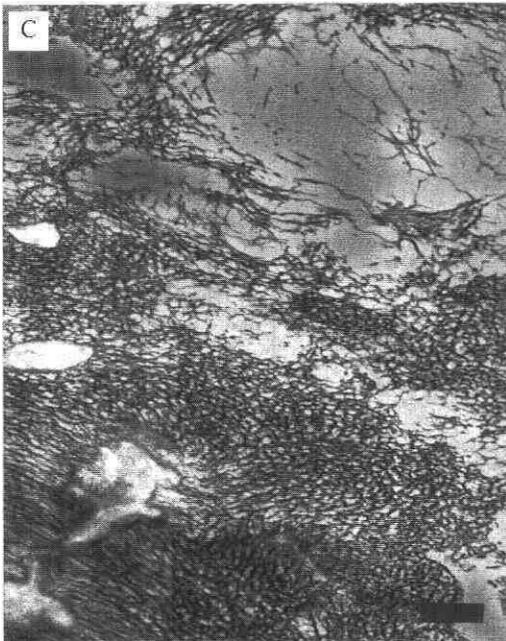
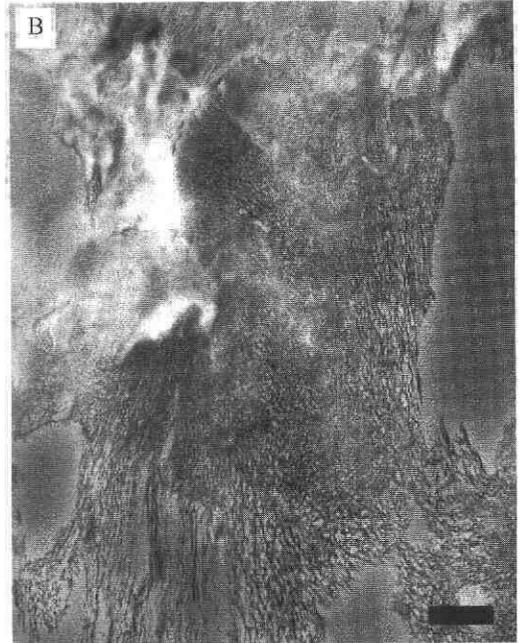


Figure 1. TEM micrograph of undegraded NR gloves (Bars = $0.5 \mu\text{m}$).



*Figure 2. TEM micrograph of NR gloves in sandy and clayey soils.
A and B: 12 and 24 week-old samples in clayey soil;
C and D: 12 and 24 week-old samples in sandy soils. (Bars = 0.5 μ m)*

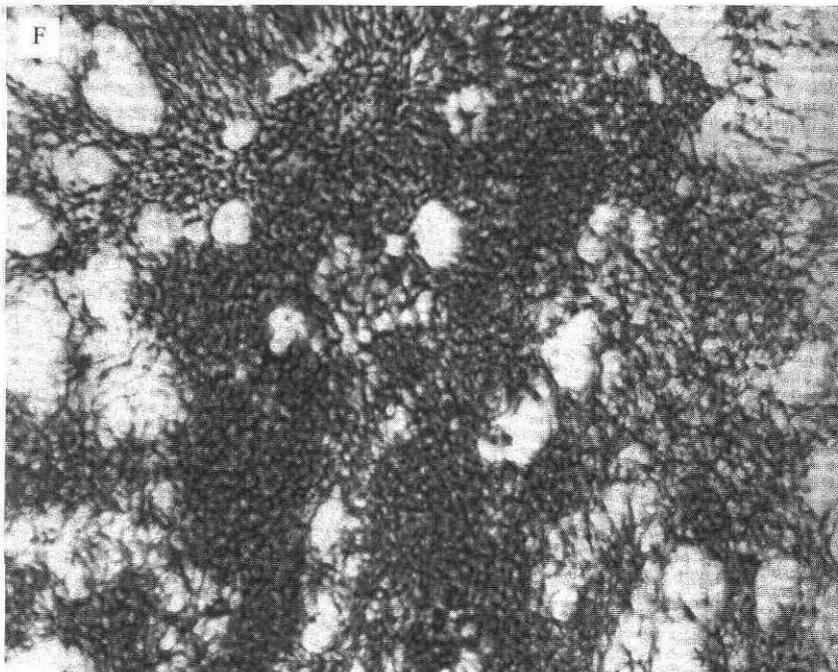
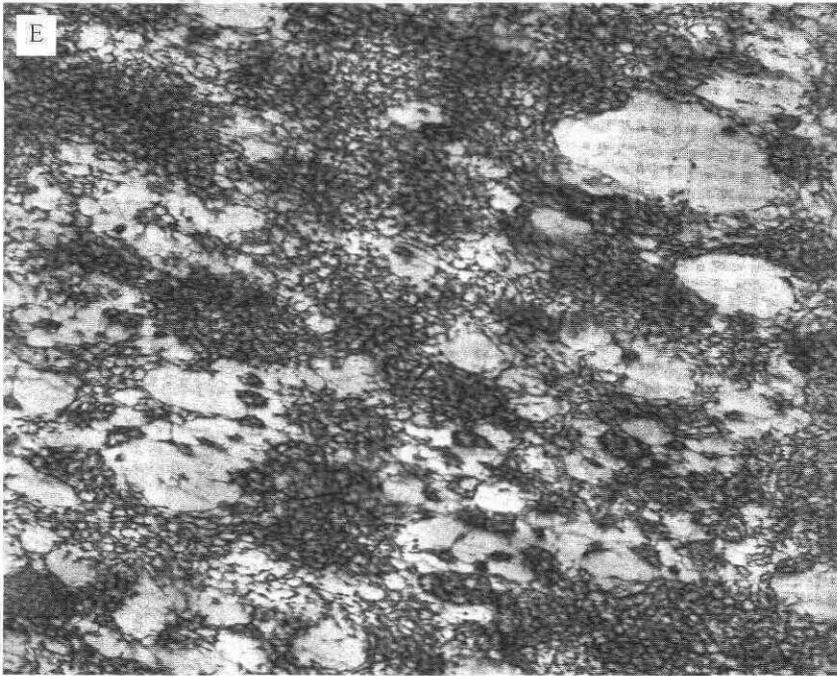


Figure 3. TEM micrograph of NR gloves in compost; E and F: 12 and 24 week-old samples. (Bars = 0.5 μ m)

Analysis by FTIR-ATR

Samples after degradation showed the appearance in oxygenated structures particularly in the region of 1780 cm^{-1} to 1630 cm^{-1} that could be attributed to carbonyl groups and at 1070 cm^{-1} to 1030 cm^{-1} that corresponded to the formation of primary alcohols (Figure 4). Disappearance of the =C-H out of plane band at 835 cm^{-1} were also observed for all the biodegraded NR gloves compared to that of the controls. These observations corresponded to oxidative chain scissions, as the formation of oxygenated structures such as ketones and aldehydes is common for oxidative chain scission in NR⁹, or could also be interpreted as oxidative reduction arising from NR biodegradation¹¹.

The results for the glove samples buried in sandy and clayey soils were similar in the trends observed, *i.e.* the relative disappearance of the *cis* double bond and the occurrence of oxygenated structures in the 24-week-old samples were appreciably higher than the 12-week-old samples. A difference was observed with the 24-week-old samples of gloves in compost, where the primary alcohols and carbonyl group bands were comparatively lower than the 12-week-old sample (Figure 5). Both degraded samples in compost also appeared to retain the band attributed to the =C-H out of plane band at 835 cm^{-1} and another major band of NR at 1450 cm^{-1} corresponding to the CH₂ deformation¹¹.

DISCUSSION

Network-visualisation of styrene-swollen samples of degraded gloves may provide a way to study the various stages of the biodegradation process since alternative physical testing methods such as tensile or swelling measure-

ments could be carried out on severely degraded NR specimens. Previous work has shown that the mesh sizes of different types of styrene-swollen dry rubbers could be correlated to the molecular weight between crosslinks as determined by stress-strain measurements¹², and that vulcanisates with a high crosslink density produce finer and smaller mesh sizes than vulcanisates with a lower crosslink density. Amir-Hashim *et al.*¹³ found the same effect when using a similar technique to investigate the effect of leaching in NR latex films.

In the present work it was found that after degradation in soil the integrity of the network structure of the glove samples was destroyed and therefore the polystyrene was able to infiltrate the latex particle microstructure. A high degree of this phase separation between the rubber and the polystyrene indicated that a high degree of degradation had taken place and that the strength of the forces maintaining the integrity of latex film had diminished. However, some areas of high density rubber network could still be observed in the samples and these presumably represented regions of highly crosslinked rubber.

The degree of phase separation between the rubber and the polystyrene that occurred was similar for the gloves degraded in the clayey and sandy soils. However, in NR gloves degraded in compost the microstructures of the latex particles were still present and visible after 12 and 24 weeks. In addition, the FTIR-ATR spectra of the 12 and 24-week-old glove samples in compost appeared to retain bands attributed to NR, namely the *cis* double bond and CH₂ deformation.

Although such results may indicate that degradation in compost was less pronounced compared to degradation in soils, the situation

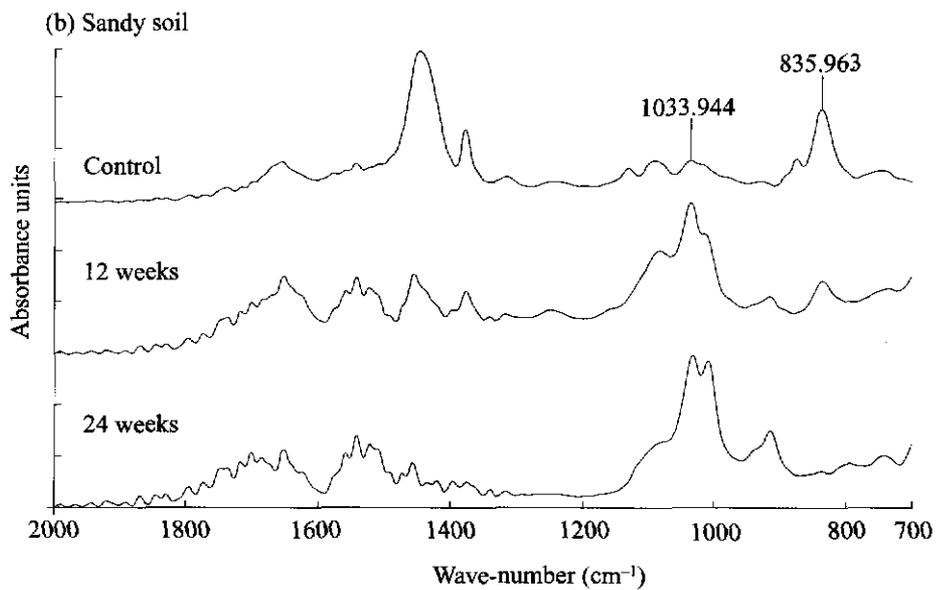
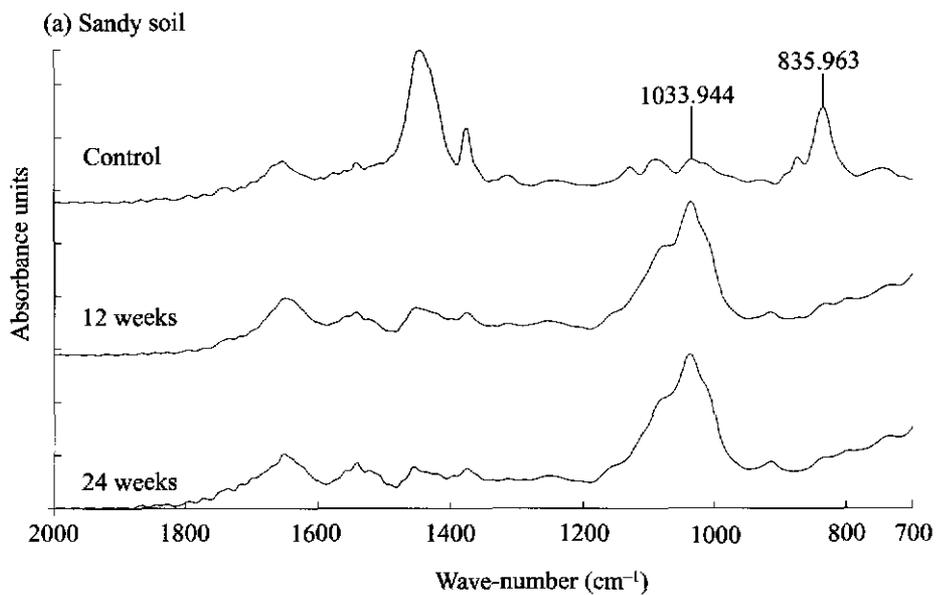


Figure 4. FTIR-ATR analysis of degraded NR gloves.

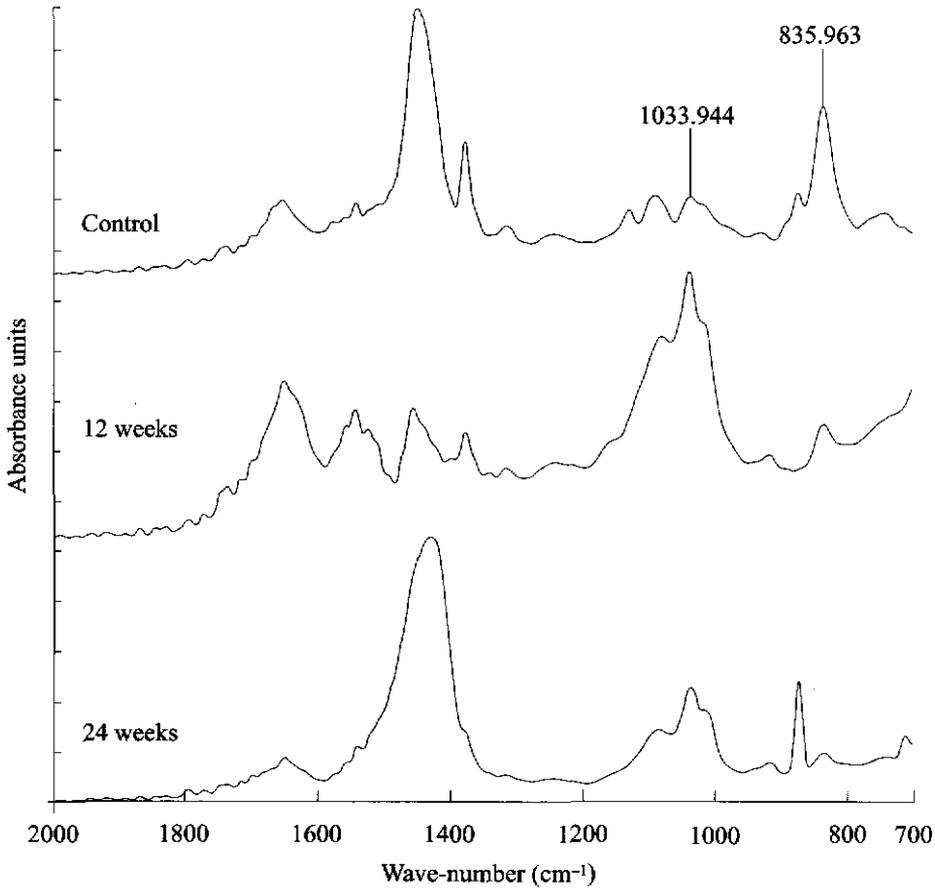


Figure 5. FTIR-ATR analysis of degraded NR gloves in compost.

was unlikely since the composting process provides favourable environmental conditions (increased microbial activity, nutrient availability, adequate oxygen, optimal moisture retention for microbial activity, early thermophilic stage) that enhance the rate of natural decomposition. Furthermore, the severity of the breakdown observed did not reflect the actual physical loss in mass of the degraded materials. For example, the sandy soil glove samples retained 21% of their initial weights (79% weight loss) at 24 weeks

compared to 57% (43% weight loss) for the clayey soil, despite their similar micrographs. Similarly, glove pieces from compost had 55% and 27% of their initial weights remaining at 12 and 24 weeks, but the state of their network mesh structure suggested an early stage of degradation.

These findings indicate that the biodegradation process was not proceeding uniformly throughout any given piece of material left to decompose. It is likely that biodegraded

shorter fragment of rubber chains were assimilated into surrounding soils, accounting for the mass losses, and leaving behind intact and highly crosslinked materials. However, the presence of microstructures of the latex particles that could still be observed in the gloves samples degraded in compost remained unclear.

CONCLUSION

The present investigation demonstrates the disruption of the visualised rubber network structures and the loss of NR *cis* double bonds following environmental degradation. It could be deduced that degradation was not progressing uniformly on gloves samples left to degrade as the visualisation of the network structures could not be correlated to the sample weight loss.

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