Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.3, 2014

# A Solvent-swelling method to visualize rubber particles network in biodegraded household Natural Rubber (NR) latex gloves

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**Abstract**
The environmental degradation of household NR latex gloves buried in soil was examined using Transmission and Scanning electron microscope. Gloves pieces were treated with acetone to remove the autooxidation formulation prior to soil burial and compared with the untreated gloves. All gloves had been allowed to degrade for various time periods (3, 12, and 18 months). Degradation degree of the samples was evaluated on the integrity of rubber network of the latex particles within the samples using electron microscope. Prior to observation under microscope, samples were prepared using a solvent-swelling method. There were marked differences between network densities of latex particles for treated and untreated samples as early as 3 month treatment. Latex particles in treated samples showed a very coarse and loose rubber network that occasionally surrounded by a network of higher density latex particles, though a few remnants of latex particles can still be seen. Whereas the untreated samples showed some distinguished latex particles membrane and the integrity of rubber network was still intact. The disintegration of rubber network became more apparent in 12 and 18 months of burial. Rubber particles network in 18 months samples disintegrated totally, and the network coalesced into free polymeric strands. Solvent-swelling method gives a clearer picture on rubber particle network.
**Key words**: solvent-swelling method, latex biodegradation, rubber particle network, household NR latex.

## Introduction

Generally the environmental degradation of carbon-chain polymers uses both abiotic and biotic chemistry to return the products of oxo-degradation to the biological carbon cycle (Scott, 1997, 2000, 2002; Billingham *et al*., 2002). Although natural rubber (NR) is a polymer "made in nature" with environmental advantages, thick latex film products appear to be bio-resistant because they take longer time to break down naturally after being discarded in waste management infrastructures. Conversely, thin latex products such as examination and surgical gloves are capable to degrade within a realistic time scale. Bacteria, fungi and the mycelium-forming actinomycetes degrade vulcanised NR products but microbial rubber degradation alone can be a very slow process (Rose and Steinbuchel, 2005; Shah *et. al*., 2013; Imai *et. al*. 2011).

The application of electron microscopy to study degradation process of NR latex is preferred for its high resolution and resolving power. Conventional transmission electron microscopy (TEM) is an imaging technique whereby a beam of electrons is transmitted through a thin specimen, then an image is formed, magnified and directed to appear either on a fluorescent screen or on layer of photographic film for viewing or to be detected by a sensor such as CCD camera for image capturing and digital archiving. Ultrathin section of polymer samples (up to 130μm in thickness) is normally required in order for the beam to penetrate and formed the image. Routine in-house method to visualize polymer ultrastructure using a TEM requires samples to be cryosectioned with a cryo-ultramictrotome, contrasted with heavy metals and visualized the sections using a TEM. However, this technique provide limited information on the interaction between the matrixes (i.e. rubber) with other components (i.e. fillers) A solvent-swelling method by mixing and polymerized styrene with NR latex films offers an alternative toward visualizing the rubber particle network and its inter-relation with other components. This paper describes initial work using the solvent-swelling procedure on degraded NR latex gloves and visualized using a TEM in an attempt to have a better understanding on the microstructure of degrading rubber particles in NR latex films.

## Materials and Methods

*Polymer swelling procedure*

The film pieces were retrieved from soils at 3, 6, 12 and 18 months after burial, and four replicates of each treatment were sampled at each sampling time. All polymer samples were styrene-swelled using the protocol as follows. Small pieces of degraded NR latex gloves were cut into small pieces (1x5 cm) and wrapped with lenses tissue. The samples were then put in a Soxhlet extraction apparatus and extracted in a refluxing acetone overnight. This was to get rid of any impurities (primarily sulphur or antioxidant) that could hinder polymerization process later on. After the extraction was completed, the samples were allowed to dry off to remove residual solvent. The samples were then immersed in a styrene solution (1 wt. % Benzoyl Peroxide, 2 wt. % Dibutyl Phthalate plasticizer) and allowed to swell in the solution for 1 – 2 days. The swollen latex strips were then cut into smaller pieces of 10 x 2 mm strips and transfer into gelatin capsule and top up the capsules with fresh styrene solution. The capsule caps were pressed firmly and labeled accordingly. The capsules were put in a sample holder and heated at 68°C in an oil bath and leaved the styrene to polymerize at least overnight. When the styrene has hardened fully, the harden block were proceed to the next step which was sectioning using an ultramicrotome.

*Sectioning of polymer with cryoultramicrotomy*

The styrene blocks were sectioned using a cryo-ultramicrotome (LKB MT-7000 ultramicrotome and CR-21 cryo-unit), with the temperature set at -110°C for the chamber, knives and the specimen. Ultrathin sections (100nm thickness, cutting speed 2mm/s) were deposited onto an uncoated copper grid (applied slightly with propanol to relax the sections and to ease the manipulation of the cryosections) in a cryo-chamber. The grids were then stained with osmium tetroxide for 1 hr in a fume cupboard. The sections were then viewed with a TEM (Philips CM12) and micrographs were recorded.

## Results and Discussion

Polymer microscopy has evolved so much recently in sample preparation especially for Transmission electron microscopy (TEM) observation. The current TEM setup which was routinely used for examining blend morphology and phase structure, swollen networks, thin films, replicas and particles has endless capability in adapting with the changes on the sample preparation methodology. Our routine in-house polymer methodology required the sample to be subjected to sub-frozen condition during sectioning and viewing the thawed sections with the TEM. This method however offered limited information on the interaction between polymer matrixes. As an example, there was hardly anything resembling a rubber particle that can be seen from the micrograph (Figure 1a) . Rubber phase was observed as gray background (unstained) and electron dense sheet and dark agglomerates were the filler. In contrast with the same sample that was styrene swelled, the demarcation of rubber particles and its network was very obvious (contrasted with osmium tetroxide) (figure 1b).

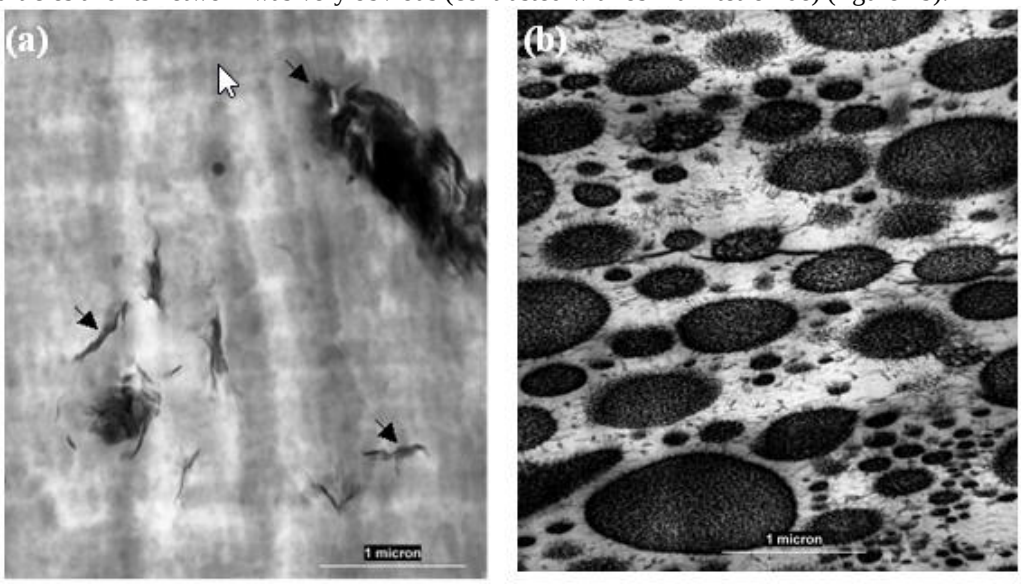


**Figure 1**: TEM micrographs of PVA latex with montmorillonite (MMT). Unswollen samples (a), styrene-swelled sample (b). Arrows indicate the fillers (MMT)

This technique was then applied to the degraded NR latex gloves to see the degree of rubber particles disintegration, the effect that was reported caused by the soil microfloras (Ikram *et al*., 2001; Scott, 1997; Ismail *et. al*., 2013). In styrene-swelled samples, rubber particles which has been stained with osmium tetroxide appeared electron dense as compared to the clear and lighter (unstained) area which are mainly polystyrene. This observation was due to a polystyrene phase that was created which was immiscible with the rubber during polymerization. Thus a phase separation occurs in which rubber chains were forced together between the growing domains of polystyrene, resulting in a mesh structure of rubber threads that was visible under the electron microscope [3].

Figure 2 showed NR gloves that were buried in the soil for 3 months. The micrographs of degraded glove (untreated samples) showed latex particles as a homogenous network structure, entangled with each other. There were areas where the latex particles stained darker than the others. It was probably due to the difference in particle density or different level at which the particles was sliced during sectioning. If the section was close to the rubber membrane, which normally comprised of lipid bi-layer, that area will stain more with osmium. Treated degraded glove (T – 3 month) showed a less prominent shape and disorganized mesh structure of rubber particles, with rubber network loosely orientated (figure 2b). Voids or polystyrene areas were more prominent as compared to the untreated samples. This inhomogeneous nature of the rubber crosslinking in treated glove, probably could be explained by the dissolution or rubber chain breakdown by microbial activities.

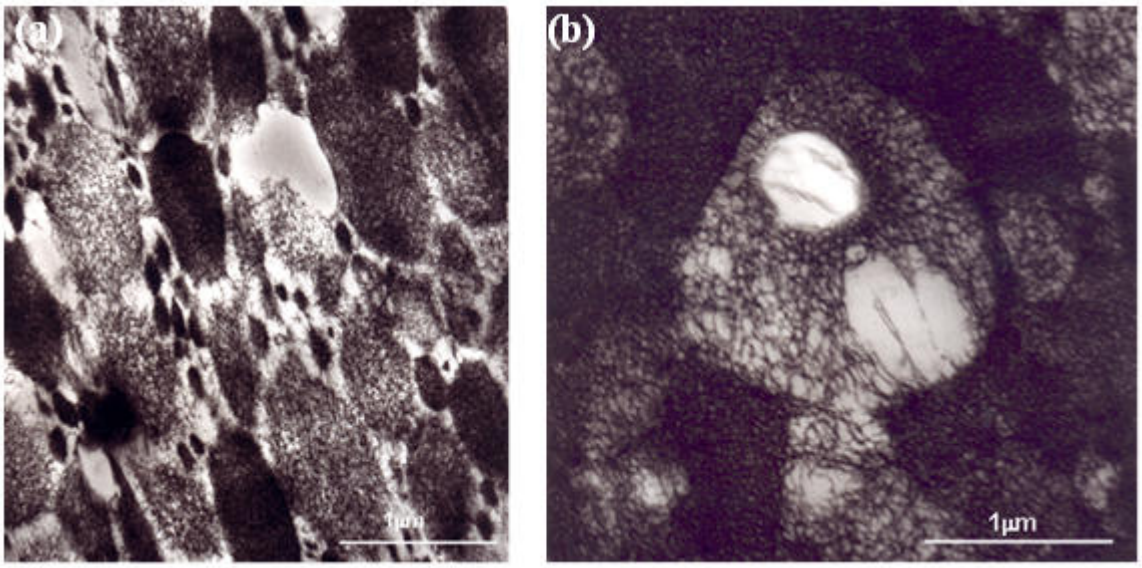


**Figure 2** : TEM micrographs of 3 months-degraded NR gloves. Darker area is a matrix of rubber particle in a polystyrene background (grayish colour). Rubber particles are still intact in untreated sample with clear demarcation of individual particles (a). Small degree of rubber particle breakdown can be seen in treated sample (b).

The rubber network between the three and twelve months-untreated sample was quite similar and not much difference in their particle integrity as shown in figure 1a and 2a. However it was very clear the disintegration of rubber particles were more apparent in the treated NR gloves. It was hardly any delineation of any individual rubber particles observed. The rubber particles appeared as a mass of entangled network, interweaving with each other in the gray matrix of polystyrene (figure 3b).

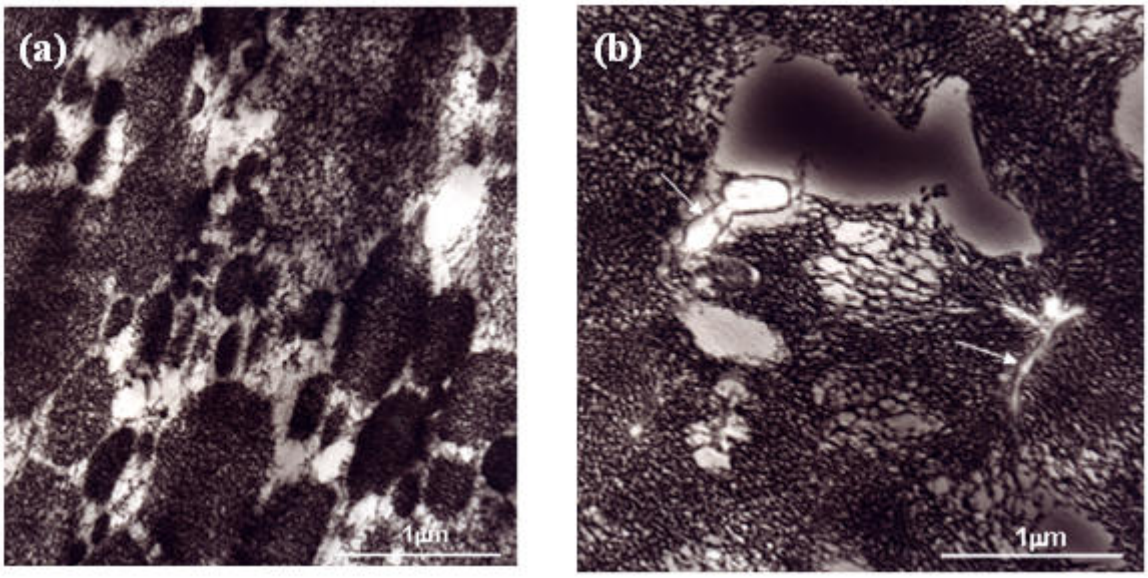


**Figure 3:** TEM micrographs of 12 months-degraded NR latex glove. Untreated NR latex glove shows extended degree of rubber particle disintegration. Some particles appear darker than the others, especially the smaller particles. Total dissolution of rubber particles in treated gloves is evidence at this stage (b). Some inclusion of microorganism can be seen in the rubber mass (arrows).

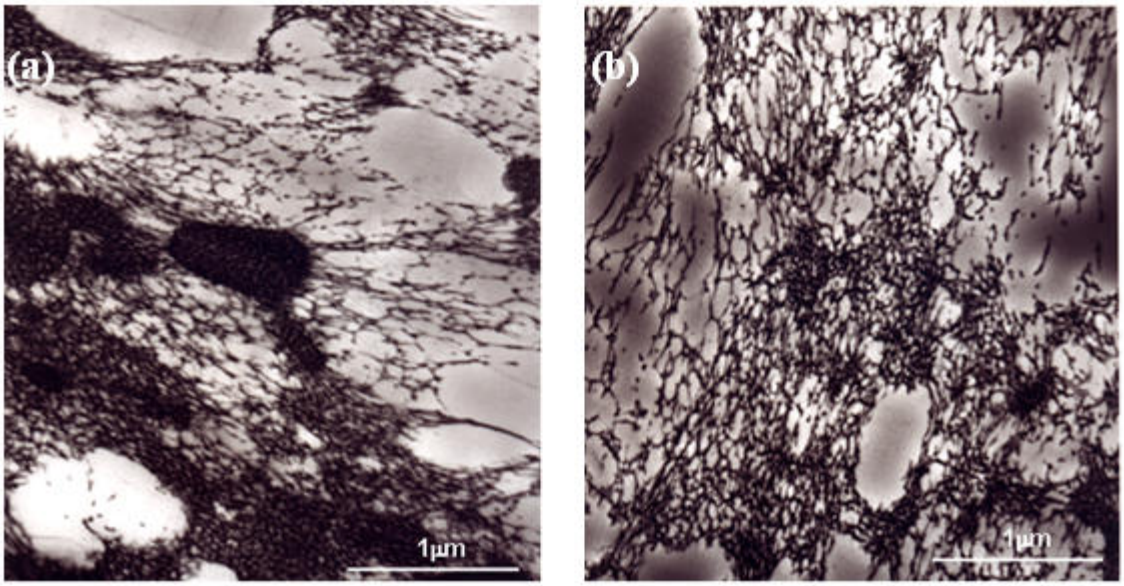


**Figure 4:** TEM micrographs of 18months-degraded NR latex gloves. A complete breakdown of rubber particles for both untreated (a) and treated (b) NR gloves.

Figure 4 of 18months-degraded gloves showed a total breakdown of rubber particles. Rubber networks became sparse in intensity and loose rubber strands mixed and blend with the polystyrene with the later has a higher concentration ratio especially in the treated sample (figure 4b). There was hardly any remnant of microorganism inclusion in the rubber matrixes as compared to the unswollen sample (Figure 5 & 6). This probably due to the rigorous process particularly during extraction steps, which removed some traces of microorganism in the rubber matrixes.

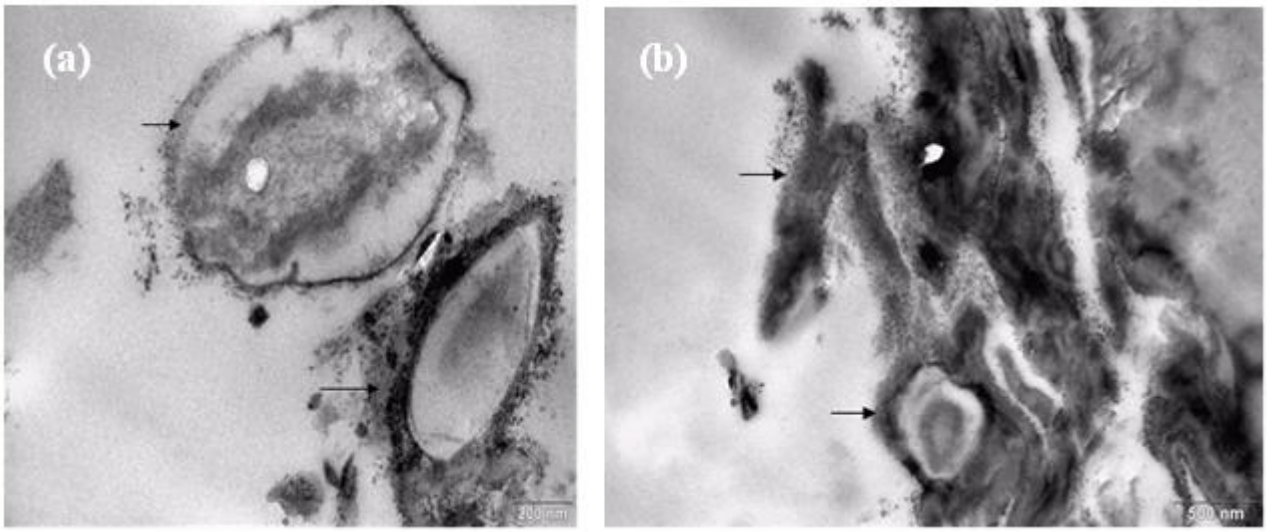


**Figure 5:** TEM micrographs of 12 months-degraded NR latex gloves. Unswollen sample. Dark inclusions in the gray rubber matrix are remnant of fungi bodies that penetrate into the interior of the NR latex film (arrows). These observations are very prominent in unswollen sample as compared to the styrene-swelled samples.

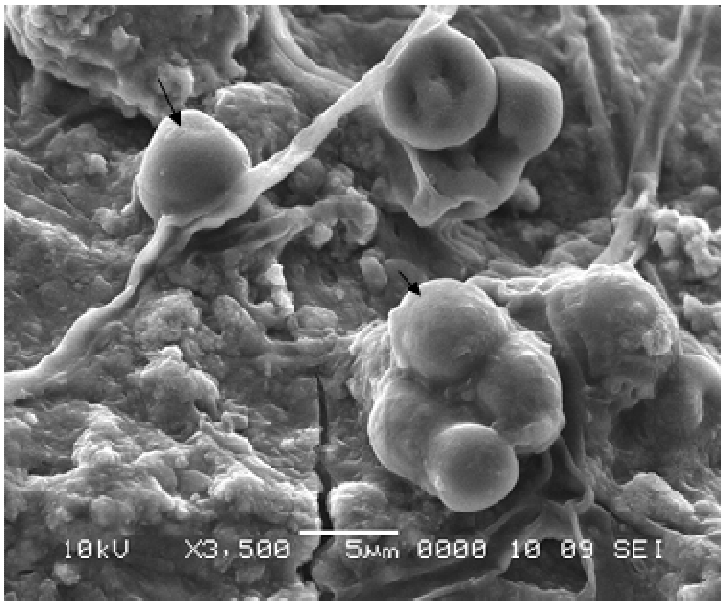


**Figure 6:** A Scanning Electron microscopy (SEM) image of microflora that resides on the surface of the degraded NR latex Gloves (arrows). A crack on the surface of the glove is also an evidence of degradation in process.

## Conclusion

Rubber particle's network in degraded NR latex films was clearly demonstrated using this styrene-swelling method. Rubber network disintegrated and clearly broke down when kept longer in the soil. The surface colonizing microorganisms may not reflect the true population densities associated with the degrading material, since much of it could have penetrated into the decaying pieces and were not liberated into the extraction media unless the pieces were blended

**Acknowledgements**

The author thanks staffs at Material Characterizatin Unit of Tun Abdul Razak Research Center, Brickendonbury, UK for capable research assistance and Dr. S. Cook for suggestions in the preparation of this manuscript. The author also acknowledges contribution by Dr. Ahmad Ikram from the Rubber Research Institute of Malaysia for supplying the latex samples. This research was funded by Fundamental Research Grant Scheme (FRGS/1/2013/ST03/UMT/02/01)

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