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ORIGINAL CONTRIBUTION

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Direct imaging and elemental mapping of microgels in natural rubber particles

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Abstract Elemental distribution maps of *Hevea brasiliensis* natural rubber gels have been obtained using electron energy-loss spectroscopy imaging in a low-energy (80 kV) electron spectroscopy imaging transmission electron microscope. Two types of gels have been investigated: a microgel contained within the natural rubber particle, and a macrogel prepared by equilibrating dry natural rubber in toluene. Both types of gels are found to contain a high amount of calcium. The intraparticle microgel is dense and rich in calcium but poor in nitrogen, indicating the predominant role of calcium in cross-link formation. The macroscopic gel is inhomogeneous, with dense calcium-rich microgels interspersed in a matrix of a less dense gel. The significant level of nitrogen associated with the matrix of the less dense gel supports the role of proteinaceous materials in the formation of the macroscopic gel.

Keywords Natural rubber particles · Microgel · Electron energy-loss spectroscopy imaging · Elemental distribution map

Introduction

Although natural rubber hydrocarbon from *Hevea brasiliensis* is predominantly composed of *cis*-1,4 polyisoprene chains, it is also known that natural rubber contains a sol and a gel fraction. These fractions are obtained when a dry natural rubber film is equilibrated in an organic solvent for polyisoprene. The soluble linear chains form the sol fraction, while the cross-linked chains form the insoluble gel fraction. It has been reported that the natural rubber gel obtained by this method shows some unusual characteristics [1]. Firstly, the mass fraction of the gel is solvent-dependent, suggesting that not all cross-links are covalent in nature. Secondly, a higher amount of nitrogenous material, presumably from proteins, is found to be associated with the gel phase compared with the sol. The gel content is also found to increase with aging of the latex. The fact that latex from virgin trees contains an extraordinarily high gel content, and that subsequent consecutive tapping for several days reduces the gel content, suggests that cross-linking commences in the rubber particle that is still inside the tree [2]. These findings have prompted considerable interest in understanding the nature of natural rubber gel and the type of cross-links involved.

So far, all reported studies have supported the role of proteins in gel formation: a higher amount of nitrogenous material associated with the gel phase compared with the sol phase [1], and a decrease in gel content in deproteinized natural rubber latex [3, 4]. It is believed that cross-linking takes place by polar interaction between proteinaceous materials and oxygen groups on the polyisoprene chains. Thus, addition of polar cosolvents to suppress the polar interaction is found to reduce the gel content [1, 4]. Physical cross-links between proteins and oxygen groups on polyisoprene chains have also been supported by Fourier transform IR spectroscopy studies [5].

All past studies on natural rubber gels were performed on macroscopic gels, where a dry rubber film is equilibrated with a solvent for polyisoprene and where the macrogel constitutes the insoluble phase. On the other hand, no studies have been reported on individual rubber particles that show direct evidence of the microgel within. The characteristics and type of cross-link involved in such a microgel, being inside the particle and excluded from hydrated membrane proteins in the aqueous phase, may be different from those of macroscopic gels. In this study, we examine individual natural rubber particles and the microgel contained within, using analytical electron microscopy imaging. This technique provides information on morphological features together with the elemental distribution at high spatial resolution. Previous characterizations of natural rubber latex film using this technique have revealed a wide range of elements present in this system [6]. Furthermore, the technique does not require special chemical treatment of the sample, such as staining, and is therefore well-suited for in situ characterization of a microgel inside the dry rubber particle. Results from the microgel are compared with those obtained for a macroscopic gel prepared by solvent extraction. The present study thus aims at providing a better understanding of microgels, the type of cross-link involved, and the relationship between microgels and macrogels in natural rubber.

Experimental

Materials

Natural rubber latex from the RRIM 600 clone of *H. brasiliensis* was collected at the Instituto Agronômico de Campinas. The latex was centrifuged at 5 °C at 10,000 rpm for 2 h. The upper fraction containing cream rubber particles was redispersed in deionized water; the final dispersion contained about 30% of solid content by weight. Natural rubber latex containing ammonia was also obtained and was centrifuged at 5 °C at 10,000 rpm for 2 h. The redispersed upper fraction contained 0.7% of ammonium hydroxide by volume. All samples used in this study were stored in a refrigerator for 18 months.

Methods

Individual particles and microgels

For microscopy imaging of individual rubber particles and microgels, 20 μL dilute dispersions (less than 0.1% by weight) of natural rubber latex were deposited onto carbon-coated parlodion films supported on 400-mesh copper grids and were dried at room temperature.

Macroscopic gels

An ammonia-preserved natural rubber latex dispersion (2 mL, about 30% by weight) was deposited on a Teflon plate and dried at room temperature. The dry film was placed in a test-tube and equilibrated in 10 mL toluene for 48 h. The tube was then centrifuged at 3,000 rpm at 20 °C for 1 h. The sol fraction formed the upper phase and the insoluble gel the bottom phase. The gel content was determined to be about 41% by weight. After removal of the sol phase, the gel was swollen with an additional 3 mL toluene. One drop of this swollen gel was placed on a carbon-coated film supported on a 400-mesh copper grid and was dried at room temperature.

Analytical electron microscopy imaging

Electron energy-loss spectroscopy imaging was carried out in a low-energy (80 kV) electron spectroscopy imaging (ESI) transmission electron microscope (TEM). Elemental mapping was performed using a Carl Zeiss CEM 902 TEM (80 kV) equipped with a Castaing–Henry energy filter spectrometer. Characteristic energy losses from interaction of electrons with various elements were selected with an energy slit of 15 eV for C (303 eV), O (544 eV), N (410 eV), Ca (358 and 370 eV), S (200 eV) and P (153 eV). These energy selections were used to create the electron energy-loss spectra which were used to produce elemental maps generated by ESI.

Results and discussion

Bright-field TEM images of nonammoniated rubber particles dried under atmospheric conditions are shown in Fig. 1. Several morphologies can be seen: spherical, pear-shaped and paramecium-shaped. Such diverse particle morphology appears to be present mostly in aged rubber latex; for freshly tapped latex, our past studies have shown that most of the particles are spherical [7]. In Fig. 1b, some small empty globules can also be seen. These are probably small particles containing low molecular polyisoprene chains that have vaporized, leaving behind empty envelopes. In all particles, two distinct regions can be seen: a light and a dark region, the latter representing a denser and/or thicker material. Figure 1d shows a bright-field image of a pear-shaped particle containing two dense regions: a large region at the base, and a smaller region at the tip of the particle. The corresponding plasmon image at 20 eV and the C distribution map are shown in Fig. 1e and f, respectively. The C map shows that the large dense

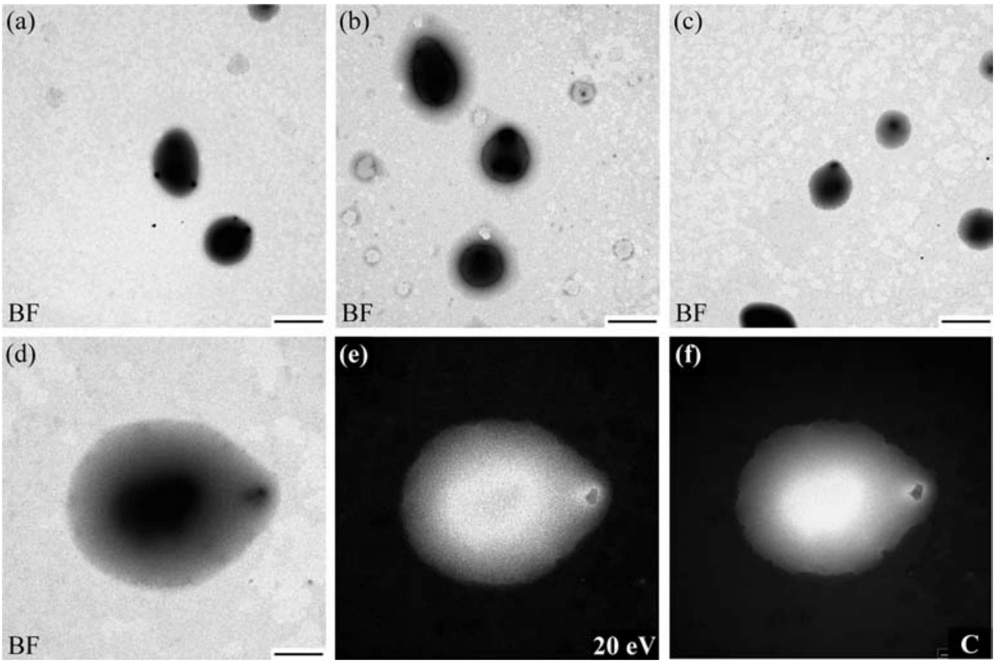

Fig. 1 Electron spectroscopy imaging transmission electron microscope (*ESI/TEM*) images of natural rubber particles stored in the presence of ammonia: **a**–**c** bright-field images showing several particles with various shapes (*scale bar* 1,500 nm); **d** bright-field image of a pear-shaped particle (*scale bar* 500 nm); **e** corresponding plasmon image; **f** C map

region at the center of the particle is proportionately rich in C, while the small region at the tip of the pear-shaped particle is less rich in C.

Since carbon arises principally from the polyisoprene chains in the hydrocarbon core, these results indicate an inhomogeneous distribution of polyisoprene within the dry particle. In *H. brasiliensis*, the hydrocarbon chains are predominantly *cis*-1,4 polyisoprene, the T_g of which is about −70 °C [8]. From this low T_g value, the particle is expected to be spread out completely to a pancake shape when dried at room temperature. Instead, uneven or incomplete spreading is observed, and our past studies [7] have also shown that the rubber particles are spread out to a fried-egg shape rather than a pancake shape. The dense unspread carbonaceous region therefore indicates the existence of a relatively immobile hydrocarbon fraction inside the particle. We propose that this dense immobile region constitutes cross-linked polyisoprene chains that form microgel within the rubber particle, denoted here as an intraparticle microgel. Such direct observation of an intraparticle microgel has not been reported in past microscopy studies on rubber particles probably because the particles in those studies had been fixed with osmium tetraoxide [9] or hardened with bromine [10, 11]. In the present study, owing to the contrast achieved in spectroscopy imaging, the sample was allowed to dry without fixing or hardening agents. The mobility of the linear chains of *cis*-1,4 polyisoprene causes the particle to spread, exposing and facilitating a direct observation of the immobile microgel. Note that the nonspherical shape of the dry particle appears to be related to the extent and distribution of the microgel phase within the particle.

TEM images of a rubber particle from a latex sample that had been stored in ammonia are shown in Fig. 2. An electron-dense membrane is clearly visible on this particle. The inhomogeneous hydrocarbon core with a dense microgel region is also evident. Thus, most rubber particles, whether stored in the absence or presence of ammonia, contain microgels in various amounts. Interestingly, the Ca distribution map (Fig. 2d) shows evidence of an extremely high level of Ca associated with the microgel. Some O is distributed throughout the particle, with a slight accumulation in the microgel region (Fig. 2e). O may be attributed with oxygen groups associated with the polyisoprene chains [12, 13, 14, 15] and also with the phospholipid–protein membrane, although in this case, the level of other elements from the membrane, such as P, S and N, is extremely low and barely detectable (images not shown). Note a small C-poor feature at about 10 o'clock on the particle rim; rich in Ca and O, this feature represents an inorganic component associated with the rubber particle. More detailed results and discussions of similar inorganic components will be presented in a future article. Elemental distribution maps obtained for other particles show similar Ca-rich microgels, with no evidence of N accumulation. These results provide evidence of ionic or covalent Ca-linkage, rather than hydrogen bonding of proteinaceous materials, as the major cross-link mechanism.

TEM and elemental maps of a macroscopic gel extracted from toluene are shown in Fig. 3. The bright-field image shows a highly inhomogeneous gel, with dense regions interspersed in a less dense matrix. The C map shows a correspondingly inhomogeneous distribution with higher intensity in denser regions. Ca is

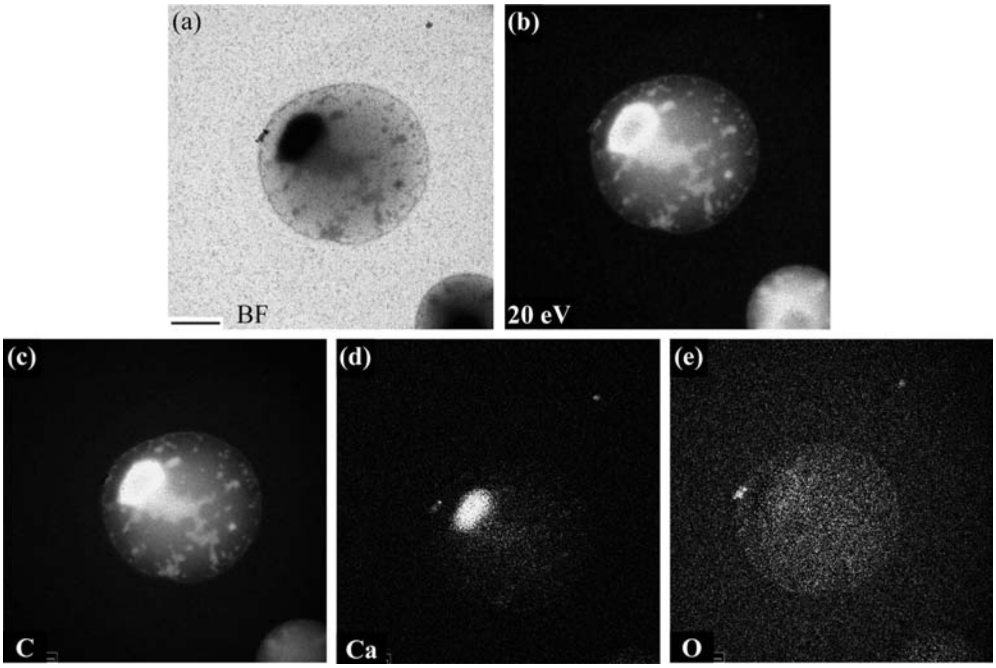

Fig. 2 ESI/TEM images of a natural rubber particle stored in the presence of ammonia (*scale bar* 500 nm): **a** bright-field image; **b** plasmon image; **c** C map; **d** Ca map; **e** O map

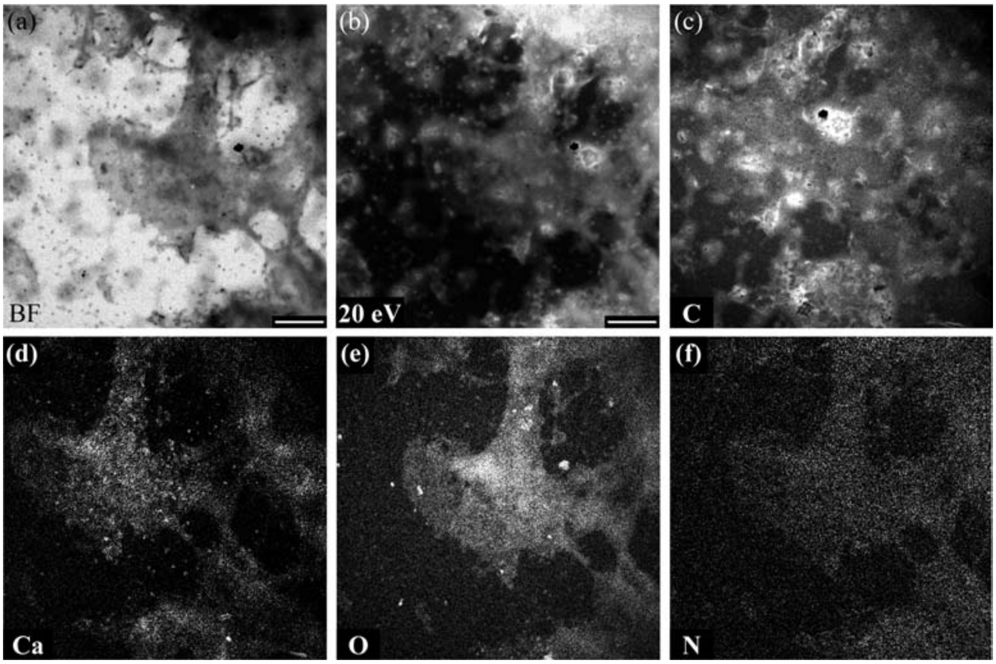

Fig. 3 ESI/TEM images of a macroscopic gel of natural rubber latex extracted from toluene (*scale bar* 500 nm): **a** bright-field image; **b** plasmon image; **c** C map; **d** Ca map; **e** O map; **f** N map. The natural rubber latex was stored in the presence of ammonia

distributed throughout the gel with a proportionately higher concentration in denser regions. Interestingly, significant amounts of O and N are also detected. O is slightly more intense in the denser regions, while N is evenly distributed with no evidence of higher accumulation in the denser regions. Although a high Ca content is seen in the case of the intraparticle microgel, the presence of significant amounts of N and O indicates the role of proteinaceous materials in the formation of the macrogel.

Thus, two types of gels are formed in natural rubber latex: a dense microgel that exists inside the rubber particle, and a macrogel that constitutes the insoluble phase when the dry rubber film is equilibrated in an organic solvent for polyisoprene. The most notable and novel result revealed from elemental imaging is the significant level of Ca associated with the microgels, providing strong evidence for covalent or ionic cross-links between Ca and integral oxygen groups [12, 13, 14, 15] or amino groups [16, 17, 18] on the polyisoprene chains.

The macroscopic gel can be described as a mixture of dense microgels from the rubber particles interspersed in the less dense matrix formed during drying of the rubber film. Interestingly, although Ca is accumulated in denser gels, it is also found throughout the less dense gel, together with high levels of O and N. The high N content is in line with the widely accepted role of proteinaceous materials in macrogel cross-links. Since the macroscopic gel was prepared from dry rubber film, it can be expected that during drying and coalescence of rubber particles further cross-linking occurs with significant involvement of protein–phospholipid membrane materials. The mechanisms of these cross-linking reactions have been suggested to involve polar interactions with protein molecules [1, 2, 3, 4, 5] and with phospholipids [4]. Reactions involving integral oxygen and amino groups have also been proposed; these reactions are also suggested to be responsible for storage hardening of the rubber [16, 18]. However, the presence of Ca and O throughout the macrogel suggests some degree of Ca–phospholipids or Ca–carboxylate cross-links as well. Indeed, evidence of an ionic Ca–carboxylate cross-link has also been found on macroscopic dry films of natural rubber latex [6].

The in situ observation of the intraparticle gel in this study provides the first direct evidence supporting early observations and hypotheses [19, 20] regarding the particulate nature and the relatively smaller size of the gel compared with those of the rubber particle. Allen and Bristow [1] later proposed that microgel formation depended on diffusion of chemical cross-link agents into the rubber particle from the aqueous serum phase. The present results show Ca to be a major cross-link agent. Allen and Bristow [1] also suggested that in dry solid natural rubber, microgel particles form a loose network by polar interaction with proteinaceous materials. This viewpoint would explain the high nitrogenous content in the macroscopic gel, the solvent-dependent gel content, and the sensitivity to polar cosolvent that can disrupt hydrogen bonding. Tanaka [17] further proposed two types of gels: a hard gel and a soft gel. The hard gel, reported to occur in excessive quantities in latex from virgin trees, cannot be solubilized after deproteinization. The hard gel thus reflects earlier references [1, 2] to a gel that is formed by diffusion of cross-link agents into the rubber particle, and that which can be formed during latex storage inside the tree. The fact that the hard gel cannot be solubilized by deproteinization can also be explained by covalent or ionic cross-links mediated by Ca as shown in our results. The soft gel referred to by Tanaka [17] thus corresponds to the fraction of the macroscopic gel that is formed by physical cross-links from polar interactions with protein molecules.

Conclusions

Elemental distribution maps for a microgel and a macrogel from natural rubber latex have been obtained using ESI/TEM. This technique is well-suited for in situ imaging of a microgel contained within the rubber particle. While the microgel exists within an individual rubber particle, the macrogel is obtained as the insoluble phase when a dry rubber film is equilibrated in an organic solvent for polyisoprene. These two types of gels differ in physical characteristics and in the type of cross-linking. The dense microgel is formed by covalent or ionic cross-links between Ca and oxygen groups attached to polyisoprene chains, while the macroscopic gel is highly inhomogeneous, containing dense microgels interspersed in a matrix of less dense gel. The high level of nitrogenous material associated with the macroscopic gel supports the significant role of proteinaceous materials in forming the less dense gel.

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