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Research paper

Mesostructure characterization by asymmetrical flow field-flow fractionation of natural rubber samples from different *Hevea brasiliensis* genotypes

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ABSTRACT

As a biopolymer produced by a tree, natural rubber (NR) entails a major drawback compared to its synthetic counterparts, namely the non-consistency or variability of its properties. One of the most important variability factors is the *Hevea brasiliensis* genotype producing the latex. The mesostructure (macromolecular structure + aggregates) of NR samples from four *Hevea brasiliensis* genotypes (RRIM600, GT1, RRIC110 and PB217) was characterized by asymmetrical flow field-flow fractionation coupled to a multi-angle light scattering (AF4-MALS) and refractive index detector (DRI). We first describe the method used to characterize NR samples by AF4-MALS from a qualitative and quantitative point of view. We focused on determining the structural parameters of the microaggregate fraction, leading to the determination of their average molar masses, size and shape. Secondly, we showed that one of the main structural differences in NR samples lies in the density of this microaggregate population. The samples from genotype PB217 contained heavier and more compact microaggregates than the other genotypes studied.

1. Introduction

Natural rubber (NR) from *Hevea brasiliensis* has unique properties, especially crystallization under strain (Ikeda et al., 2016), low crack propagation and low heat buildup (Mark et al., 2005), which have never been mimicked by synthetic rubbers. This biopolymer is used to make items such as tires, anti-vibration parts or surgical gloves. Toki et al. (2008) proposed that certain unique NR properties could be due to what they called the *naturally occurring network* of NR or, in other words, its specific *associative structure* (Vaysse et al., 2012; Rolere et al., 2016a, 2016c). Although several mechanisms have been proposed in the literature (Gan, 1996; Tanaka and Tarachiwin, 2009; Intapun et al., 2010; Wisunthorn et al., 2012), the structuration of NR is still unclear, especially the genesis of microaggregates (Voznyakovskii et al., 1996; Kim et al., 2008; Dubascoux et al., 2012; Rolere et al., 2016c) (Fig. 1) and their role in the structuration of NR, hence in its properties.

Because of its specific associative structure, when NR is put in good solvents for polyisoprene it very often exhibits an insoluble part usually called the macrogel by some authors (Allen and Bristow, 1963; Ehabe and Bonfils, 2011) and the gel phase by others (Amnuaypornsri et al., 2008; Tanaka and Tarachiwin, 2009) (Fig. 1). The biochemical composition of the NR macrogel was extensively studied very recently (Rolere et al., 2016b, 2016a; McMahan et al., 2015). On a macromolecular point of view, the composition of the soluble part is also very specific as it contains two main entities: i) linear macromolecules of polyisoprene of different lengths (random coil structure) and ii) very compact microaggregates or microgels (sphere-like structure) (Fig. 1) (Kim et al., 2008, 2009; Dubascoux et al., 2012; Rolere et al., 2016c). Links between non-isoprene compounds (lipids, proteins, and/or mineral elements) and the polyisoprene chains would appear to be the main reason for macrogel formation in NR (Grechanovskii et al., 1987; Tanaka and Tarachiwin, 2009; Gan, 1996; Rippel et al., 2005). Owing to this complex associative structure, speaking of a macromolecular structure for a biopolymer such as NR is rather reductive. The term *mesostructure* is used to cover both the macromolecular structure and the aggregates which are formed in NR (Fig. 1). In terms of aggregates (or gel), most authors have only determined the macrogel content and very few authors have used more complete methods able to determine

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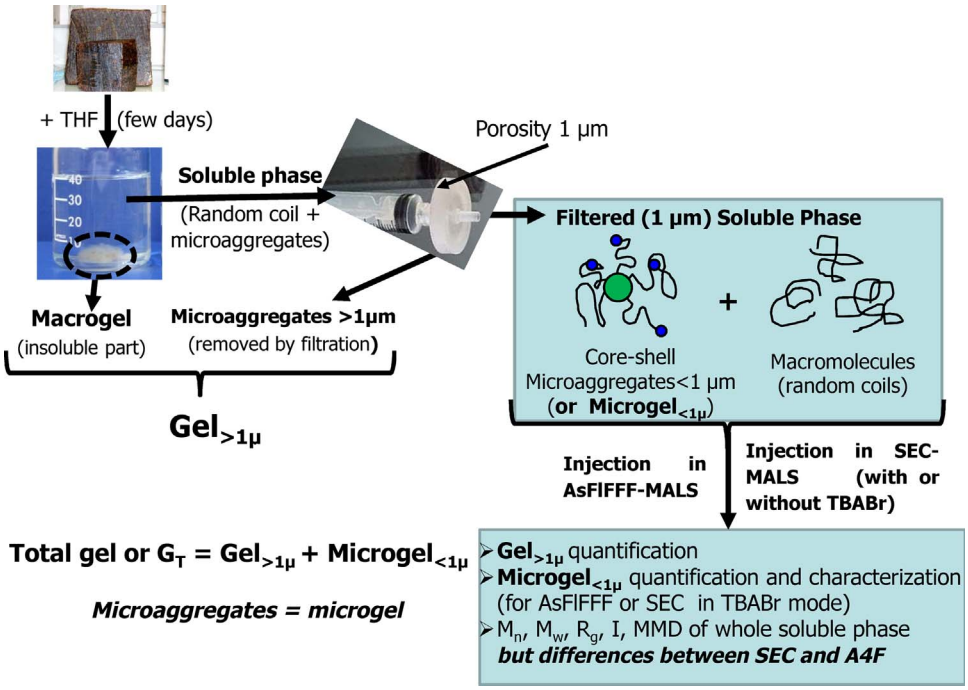

Fig. 1. Different types of aggregates (or gels) present in a solution of natural rubber in tetrahydrofuran (THF). For the proposed structure of core-shell microaggregates (Kim et al., 2008, 2009), the core can be either proteins and/or phospholipids as proposed by several authors (Grechanovskii et al., 1987; Shiibashi, 1987; Tanaka and Tarachiwin, 2009; Carretero-Gonzalez et al., 2010).

the microaggregate (or microgel) content too (Shiibashi, 1987; Fuller, 1988; Bonfils et al., 2005; Ngolemasango et al., 2003; Rippel et al., 2005). Very few studies were done on the structure of these microaggregates. Voznyakovskii et al. (1996) studied centrifuged NR solutions in toluene by dynamic light scattering. They showed that the NR solutions contained two populations of microaggregates with diameters ranging between 0.9–5 µm and 5–12 µm. Kim et al. (2008), using size exclusion chromatography coupled to a multiangular light scattering detector (SEC-MALS), showed that microaggregates, the sizes of which were below 1 µm (Microgel $_{<1\mu}$, Fig. 1), were still present in solution after filtration. Indeed, before injection in SECMALS, the solutions were filtered on 1 µm porosity filters. They showed that these microaggregates were very compact entities. Indeed, their mean radius of gyration (R_g) was about 110–130 nm and their weight average molar mass (M_w) between 4 and 12 million g mol^{-1}. This compactness was confirmed by Dubascoux et al. (Dubascoux et al., 2012) by asymmetrical flow field-flow fractionation coupled to a multiangular light scattering detector (AsFlFFF-MALS or AF4-MALS). AF4-MALS is a very versatile technique allowing the size and molar mass characterization of macromolecules and particles, especially the aggregates formed (Baalousha et al., 2011; Schimpf et al., 2000). Although, this technique is widely used with aqueous mobile phase, for rubber analysis in organic solvent only very few studies were done (Dae Young Bang et al., 2007; Makan et al., 2016).

As a biopolymer produced by a tree, NR entails a major drawback compared to its synthetic counterparts, namely the non-consistency or variability of its properties. As a consequence, some products have to be rejected, generating feedstock waste and energy losses in manufacturing processes, leading to economic and environmental costs. One of the main sources of this variability in NR properties is the genotype of the rubber tree producing the latex (Yip, 1990; Bonfils et al., 2005; Kim et al., 2010b; Lotti et al., 2012; Liengprayoon et al., 2013). Therefore, it is important to fully characterize the mesostructure of NR samples from different genotypes of rubber trees, especially the structure of microaggregates of a size inferior to 1 µm (*Microgel* $_{<1\mu}$ Fig. 1). To achieve this main objective, we characterized NR samples from different *Hevea* genotypes by size exclusion chromatography (SEC) and asymmetrical flow field-flow fractionation (AF4) both coupled to a multiangular light scattering detector (MALS).

2. Experimental

2.1. Samples

The NR samples used for this study were ribbed smoked sheet (RSS) prepared from monoclonal latex either from Thailand (genotypes RRIM600 and GT1) or from Cambodia (genotypes PB217 and RRIC110). Samples from Thailand were prepared at the plantation of Visahakit Thai Rubber Co., Ltd, Chantaburi, Thailand, in September 2011. Samples from Cambodia were prepared in the experimental station of the Cambodian Rubber Research Institute in November 2011. RSS were prepared according to the method described previously by Rodphukdeekul et al. (2008). All RSS samples were stored in a climate chamber (28 °C, 80% relative humidity) between 1 and 3 months prior to analysis by AF4 or SEC. The synthetic poly(cis-1,4-isoprene) used in this study was Kraton IR 307 (Kraton polymer, Houston, USA). Each RSS sample was homogenized (6 passes) according to ISO 1795 and ISO 2393 on a two-roll mill (BLERE I.F. 50 #1400, 51/64 model). The roll temperature was maintained at 27 °C and the nip was 1.69 mm. The speeds of the front and back rolls were 24 and 34 rpm, respectively.

2.2. Preparation of solutions for injection in SEC or AF4

The NR samples (60 ± 5 mg) were dissolved in pure tetrahydrofuran (THF, 30 mL, HPLC grade, VWR France) stabilized with 2,6-di-*tert*-butyl-4-methylphenol (BHT, 250 mg/L). After storing for 7 days in the dark at 30 °C (during the storage time, the solutions were stirred 1 h/day), the solutions were filtered (Acrodisc 1 µm, glass fiber, Pall France) and injected into a SEC-MALS (after dilution by two) or AsFlFFF system (initial concentration). Three solutions were prepared for each sample and each solution was injected once.

2.3. Determination of Gel $_{>1\mu}$

The "gel superior to 1 µm" (*Gel* $_{>1\mu}$ Fig. 1), includes all aggregates (macrogel and microaggregates superior to 1 µm) removed from the solution by the filtration before injection in SEC or AF4. The concentration (C2) of the soluble fraction after filtration was calculated from the quantity of material eluting from the columns (SEC) or the