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Analysis Method

Size-characterization of natural and synthetic polyisoprenes by Taylor dispersion analysis

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ABSTRACT

Non-aqueous Taylor dispersion analysis (TDA) was used for the size-characterization of natural and synthetic polyisoprenes (4×10^3–2×10^6 g/mol molar mass). Not only the weight-average hydrodynamic radius (R_h), but also the probability distribution of the hydrodynamic radius, were both derived from the Taylorgrams by a simple integration of the elution profile and by a more sophisticated constrained regularized linear inversion of the Taylorgram, respectively. Results in terms of size characterization (hydrodynamic radii between 2 and 100 nm) were compared to size exclusion chromatography coupled to a refractive index-based mass detector. Multimodal size distributions were resolved by TDA for industrial and natural polyisoprenes, with the advantage over the chromatographic technique that, in TDA, there is no abnormal elution of microaggregates (hydrodynamic radii ~ 40–50 nm). Considering the importance and the difficulty of characterizing polyisoprene microaggregates, TDA appears as a promising and simple technique for the characterization of synthetic and natural rubber.

1. Introduction

Natural rubber (NR) is a biopolymer produced from latex of *Hevea brasiliensis* trees. NR is used to prepare many goods, the main one being tires that absorb nearly 70% of the 12 million tons annually worldwide. As an agro-material produced by a tree, NR exhibits rather variable properties for many reasons (genotype of the tree [1,2], season [3], maturation of coagula [4–6], etc.). Thus, as for other polymeric materials used in industrial applications, it is important to characterize the NR macromolecular structure as a prelude to predicting NR end-use properties [7,8].

Natural rubber has a very specific associative structure. When dispersed in good solvents for polyisoprene, NR presents a soluble fraction and an insoluble fraction, often referred to as “macrogel” [9,10] or “gel phase” [11,12]. The composition of the soluble part is also specific as it contains two main entities: (*i*) polyisoprene linear macromolecules of different lengths, with a random coil structure, and (*ii*) very compact microaggregates or microgels (sphere-like structure) [13–15]. Links between non-isoprene compounds (lipids, proteins, and/or mineral elements) and the polyisoprene chains are thought to be the main reason for the macrogel formation in NR [12,16,17]. Although an international standard (ISO 16564) exists to characterize the NR macromolecular structure by size exclusion chromatography (SEC) or gel permeation chromatography (GPC), the development of new analytical techniques is still required to improve the characterization of NR using orthogonal or complementary methods.

This paper describes a novel method to characterize the NR macromolecular structure: Taylor dispersion analysis in organic solvent. Taylor Dispersion Analysis (TDA) is an absolute sizing method requiring no calibration and allowing the determination of the diffusion coefficient (or, equivalently, the hydrodynamic radius) of solutes of any size between one angstrom and a few hundred nanometers. TDA is based on the dispersion of a sample band in an open tubular column under Poiseuille flow. The combination of the dispersive parabolic velocity profile with the molecular diffusion of the solute leads to a specific dispersion, called Taylor dispersion. Started with the pioneering work of Taylor on macroscopic tubing [18], TDA is now implemented in narrow capillaries (typically 50 µm internal diameter), leading to much faster analysis (a few min) and requiring only tiny sample amounts (a few nL) [19–22]. In its modern miniaturized version, TDA can be very easily implemented on commercial capillary electrophoresis apparatus. Alternatively, fast TDA can be performed on HPLC equipment at a high linear velocity ($\sim 30\ \mathrm{cm\ s^{-1}}$) in a coiled open capillary tube of 250 µm i.d. × 30 m typical dimensions, as recently

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introduced by the Holyst group [23]. In the latter configuration, the genuine conditions for the applicability of TDA are not fulfilled [24], however, an empirical correction can be used to get the correct diffusion coefficient [23].

TDA was applied to the characterization of proteins [25–27], macromolecules [28–30], drug delivery systems [31,32], nanoparticles [21,33,34], polyelectrolyte complexes and polyplexes [35], pharmaceutical compounds [36,37], and micelles or microemulsions [38,39]. It can also be used for the study of molecular interactions [40,41]. The application of TDA is not limited to water-soluble solutes, as demonstrated by the size characterization of polystyrenes in THF [28], asphaltenes/bitumen in hexane or THF/acetonitrile mixtures [42,43], and drugs in pharmaceutical solvents [36]. Basically, TDA yields the weight average hydrodynamic radius when using a mass sensitive detector, such as a UV detector, for a polymer absorbing via the repeating unit [44]. This information is obtained by simple integration of the elution temporal profile (also called Taylorgram). However, we recently demonstrated that more sophisticated data analyses allow one to determine the full hydrodynamic radius distribution, thus providing valuable information on the sample polydispersity [45,46].

In this work, we demonstrate that TDA can be used to get, within a few minutes, the hydrodynamic radius distributions of commercial natural and synthetic polyisoprenes. The data obtained by TDA are compared to those derived from size exclusion chromatography (SEC) coupled to multi-angle static light scattering (MALS).

2. Experimental

2.1. Materials

The two NR samples used for this study were all TSR (Technically Specified Rubber). Sample NR1 was industrial TSR10 grade prepared by natural coagulation of latex followed by several days of coagulum maturation before processing. This sample was treated with neutral hydroxylamine sulfate (NHS) to stabilize its properties during storage, yielding a TSR10CV grade (CV stands for constant viscosity), a special grade not prone to storage hardening. Sample NR2 was prepared by acid coagulation (formic acid) of fresh field latex. Neutral hydroxylamine sulfate (NHS) was added to the latex prior to coagulation to inhibit storage hardening of rubber [47]. Sample NR2 was TSR5CV grade. Three synthetic industrial *cis*-1,4-polyisoprenes (PI) were used directly as received: IR2200 (NatSyn 2200, Goodyear chemical), IR307 and IR309 (Kraton polymers). Standard linear polyisoprene (PI) of 4.5, 22, 111, 307, 361, 608 and 766 × 10^3 g/mol weight-average molar masses were obtained from Polymer Standard Service (PSS, Germany) and were used as received.

2.2. Determination of the number- and weight-average molar mass by size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS)

For all samples, the macromolecular structure was characterized by SEC-MALS, except for standard polyisoprene, for which the number average, M_n, and mass-average, M_w, molar mass were given by the supplier. The samples (30 ± 5 mg), from NR pellets, were dissolved in tetrahydrofuran (THF, 30 mL, HPLC grade, VWR France) stabilized with 2,6-di-*tert*-butyl-4-methylphenol (BHT). The solutions were stored for 7 days in the dark at 30 °C (during storage, the samples were stirred for 1 h/day), they were then filtered (Acrodisc 1 µm, glass fiber, Pall France) and finally injected into the SEC-MALS apparatus. For each sample, three solutions were independently prepared and measured. The SEC equipment comprises an online degasser (EliteTM, Alltech), a Waters 515 pump, a refractive index detector (Waters 2410) and a multi-angle light scattering detector (Dawn DSP, Wyatt Technology). Two Waters HMW6E columns (Styragel HMW columns packed 20 µm particles, 300 mm × 7.8 mm I.D.), maintained at 45 °C, and a guard column (same stationary phase as the two separation columns) were used. The mobile phase was stabilized THF injected at a flow rate of 0.65 mL min^{-1}; the injected volume was 100 µL. The MALS detectors at all 18 angles were calibrated using a THF solution of a polystyrene standard with low polydispersity (M_w = 30.3 kg mol^{-1}, Wyatt technology). The same solution was used to determine the interconnection volume between the two detectors (0.235 mL). The basic theory of determining the weight-average molar mass and radius of gyration for a dilute solution of a macromolecule is well known and described in numerous papers in the literature [48,49]. The weight average molar masses and radius of gyration at each slice of the chromatogram were calculated using the Berry method for extrapolation, as implemented in ASTRA software (version 5.3.1, Wyatt technology). The order of polynomial fit for the Berry analysis was two. Twelve angles, from 38.8° to 138.8°, were used for the analysis. The differential refractive index increment at λ = 633 nm was dn/dc = 0.130 mL g^{-1} [50].

2.3. Determination of the weight-average hydrodynamic radius R_h and of the R_h distribution by Taylor dispersion analysis

TDA experiments were performed on a PACE MDQ Beckman Coulter (Fullerton, CA) apparatus. Capillaries were prepared from bare silica tubing purchased from Composite Metal Services (Worcester, United Kingdom). The capillary dimensions were 40 cm (30 cm to the detector) × 50 µm I.D. New capillaries were conditioned with the following flushes: 1 M NaOH for 30 min and water for 10 min. Before sample injection, the capillary was filled with cyclohexane (8.62 × 10^{-4} Pa s viscosity). Between two TDA analyses, the capillary was flushed with cyclohexane (30 psi for 2 min). A mobilization pressure of 1 psi (~71 mbar) was applied with cyclohexane vials at both ends of the capillary, resulting in a flow rate of 184 nL min^{-1}. Samples were dissolved in cyclohexane at a concentration of 1 g/L. Sample injection (2.8 nL) was performed hydrodynamically on the inlet side of the capillary (0.3 psi for 3 s; ~1% of the capillary volume). Solutes were monitored by UV absorbance at a wavelength of 200 nm. The elution time was systematically corrected for the delay in the application of the pressure (pressure ramp time, 15 s) by subtracting 7.5 s (half-time of the pressure ramp) to the recorded elution time. The temperature of the capillary cartridge was set at 25 °C.

Two different methods were used to determine the weight-average hydrodynamic radius by TDA. The first method (*method 1*) is based on the integration of the Taylorgram [22], and the second method (*method 2*) is based on a deconvolution of the Taylorgram using a recently published Constrained Regularized Linear Inversion (CRLI) method [45].

In method 1, the average diffusion coefficient D is obtained by integration of the Taylorgram (or temporal elution profile) in order to calculate the temporal variance of the Taylorgram, σ^2. The integration of the elution profile was performed using the discrete form of the following equation [22]:

$$\sigma^2 = \frac{\int S(t)(t-t_0)^2 dt}{\int S(t) dt} = \frac{\sum_{i=n}^{m} S_i (t_i - t_0)^2 (t_{i+1} - t_i)}{\sum_{i=n}^{m} S_i (t_{i+1} - t_i)} \quad (1)$$

where $S(t)$ is the detector response, t_i is the elution time for a given point i of the Taylorgram and t_0 is the average elution time. n and m are the starting and ending points that are considered for the integration of the Taylorgram.

The integration of the Taylorgram was only performed on the left part of the elution profile (the end point was t_0), before and after subtracting the Gaussian contribution due to the small molecules (see results and discussion section). Corrections of the temporal variance and average detection time due to the injected volume were also taken into account, as described elsewhere [29], but these corrections are negligible when the injected volume is ≤ 1% of the capillary volume to the detection point [22], as is the case here. Finally, the so called Taylor