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Characterization of natural rubber using size-exclusion chromatography with online multi-angle light scattering
Study of the phenomenon behind the abnormal elution profile

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ABSTRACT

Natural and synthetic poly(*cis*-1,4-isoprene) were characterized by size-exclusion chromatography coupled with an online multi-angle light scattering detector (SEC–MALS). Unlike synthetic poly(*cis*-1,4-isoprene) (SR), natural rubber (NR) samples showed anomalous elution profiles. The beginning of elution was very similar to SR but, after a certain elution volume, the molar masses of the eluting macromolecules increased with elution volume instead of continuing to decrease, which resulted in an upturn curve profile. Adding tetrabutylammonium bromide (TBABr) to THF (solvent and mobile phase) removed this phenomenon. In addition, using different concentrations of TBABr showed that TBABr had two simultaneous actions. TBABr reduced the abnormal elution profiles and the quantity of aggregates (insoluble part or gel). These results mean that the main phenomenon involved in abnormal elution was delayed entities adsorbing on the column packing. Their delayed elution was responsible for the artificial increase in molar masses, especially at high elution volumes. The results obtained suggest that these entities are very compact and have a sphere-like structure.

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1. Introduction

Natural rubber from *Hevea brasiliensis* in a good solvent, for poly(*cis*-1,4-isoprene), always leads to an insoluble fraction, the gel (associations between poly(*cis*-1,4-isoprene) chains), and a soluble fraction, with more or less branched poly(*cis*-1,4-isoprene) macromolecules. The gel in NR lies behind associations between poly(*cis*-1,4-isoprene) chains due to junctions by non-isoprene compounds (proteins [1,2] and lipids [3]).

Subramaniam [4] was the first to characterize the macromolecular structure of NR by size-exclusion chromatography (SEC). Subramaniam [4] highlighted the clonal variability of the inherent macromolecular structure of NR, just after tapping. The inherent molar mass distributions (MMD_0) differed from one clone to another, as did the properties of raw NR once processed [5,6]. NR from *Hevea brasiliensis* has a very high weight-average molar mass (M_w) ranging from 500 kg/mol to more than 1000 kg/mol depending on the samples studied [7,8]. Today, for a more complete characterization of NR macromolecular structure, SEC–MALS is a very versatile technique since the multi-angle light scattering (MALS) detector provides absolute M_w, along with the radius of gyration ($\langle s^2 \rangle^{1/2}$ or R_g) throughout the chromatogram. This combination of M_w and a size parameter (R_g), in the case of polydisperse polymers, can be used to obtain information about the shape of polymer chains (Flory exponent, ν, Eq. (1), also known as the scaling parameter [9]) and the distribution of branching [10].

$$R_g = AM_w^{\nu} \quad (1)$$

No studies by SEC–MALS on the structure of NR from *Hevea brasiliensis* have been reported to date, unlike many other polymers, be they synthetic [11–13] or natural [14–16]. Only Bushman et al. [16] mentioned characterization by SEC–MALS on natural rubber from two lettuce species giving no chromatogram but only M_w (1270–1382 kg/mol), radius of gyration (69–80 nm) and Flory exponent values (0.36–0.43). The method used for extrapolation at zero angle was not given.

This work presents the study of the structure of samples of natural and synthetic poly(*cis*-1,4-isoprene) using SEC–MALS. Three

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different model samples of natural rubber (NR) and two commercial synthetic poly(*cis*-1,4-isoprenes) (SR) (Natsyn2200, IR305) were analyzed. The study was focused on understanding the abnormal elution observed for raw NR without any purification, and to identify the phenomenon behind it. Indeed, our main objective is to develop a SEC–MALS characterization of raw industrial NR to link structure parameters to rheological behaviour. Finally, an additional study on influence of filters porosity, used to filter solutions before injection in SEC, is presented.

2. Experimental

2.1. Materials

The NR samples used for this study were all TSRs (Technically Specified Rubber) (Table 1). They were prepared in a rubber processing factory in Cambodia. All the grades were prepared from fresh latex, except for TSR10 grade. The latex was coagulated at pH 5.2 with formic acid and allowed to coagulate for 16 h. The coagulum was fed into a crusher, then to three roll crepers and finally into a shredder to obtain crumbs. The crumbs were dried in an air-dryer (Li-Hoe) at 110–120 °C for 3 h. Because of the importance of clonal origin on the quality of NR [5,7], NR samples were prepared from the latex of a single clone (PR107). Sample 1SAP21 (Table 1) was a TSR10 grade prepared by natural coagulation of latex followed by 4 days of coagulum maturation before processing. Samples AJ and AN (Table 1) were prepared by acid coagulation (formic acid) of fresh field latex, it was a TSR5 grade. Samples AM and CB (Table 1) were prepared like the latter, but neutral hydroxylamine sulphate (NHS) was added to the latex prior to coagulation with formic acid. The sample was therefore TSR5CV grade (CV for constant viscosity), a special grade not prone to storage hardening. NHS inhibits the storage hardening of rubber as described by Sekhar [17].

Two synthetic poly(*cis*-1,4-isoprene) were Natsyn2200 (Goodyear chemical) and IR305 (Kraton polymers) and were used directly as received.

2.2. SEC–MALS analysis

The samples (25 ± 5 mg) were dissolved in tetrahydrofuran (THF, 40 mL, HPLC grade) stabilized with 2,6-di-tert-butyl-4-methylphenol (BHT), with or without tetrabutylammonium bromide (TBABr, Section 3). For a better repeatability, three solutions were prepared for every sample and each solution was injected once. The values of gel rates and average molar masses are the mean of the three injections for every sample. After 14 days, the solutions were filtered (Acrodisc 1 μm, glass fibre, Pall) and injected in SEC, excepted for Section 3.4 of results and discussion. In this last section, the influence of filter porosity was tested with filters of 1 μm porosity (Acrodisc 1 μm, glass fibre, Pall) and 0.45 μm porosity (Acrodisc PSF, PTFE membrane, Pall). Knowing the exact initial concentration of the sample solutions and determining the injected quantity, after filtration and elution, made it possible to determine the insoluble or gel rate.

The SEC equipment consisted of an online degasser (Elite™, Alltech), a Waters 515 pump, a refractive index detector (Waters 2410) and a multi-angle light scattering detector (Dawn DSP, Wyatt technology). The columns were two Styragel HMW 6E mixed bed columns (20 μm, 300 mm × 4.6 mm I.D.) (Waters) with a guard column. The columns were maintained at 45 °C. The mobile phase was THF, with or without TBABr (Section 3) at a flow rate of 0.35 mL/min; the injected volume was 50 μL.

All diode detectors at all 18 angles in the MALS instrument were normalized using a THF solution of low polydisperse polystyrene standard (M_w = 30.3 kg/mol, 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 was already described numerous times in the literature [11,18]. The weight-average molar masses and radius of gyration at each slice of the chromatogram were calculated using Berry method for extrapolation in ASTRA software version 5.3.1 (Wyatt technology). The order of polynomial fit used with Berry method was two. Fourteen angles, from angle 3 (32°) to angle 16 (134°), were used for calculation.

For a synthetic poly(*cis*-1,4-isoprene) in THF, the Polymer Handbook gives a differential refractive index increment (d*n*/d*c*) value equal to 0.124 mL/g at 633 nm [19]. The value we obtained with the differential refractometer Optilab DSP (Wyatt technology) was 0.130 mL/g for natural rubber; that value was used in this study for all natural and synthetic polyisoprene samples.

3. Results and discussion

For this study (Sections 3.1–3.3), three chromatographic conditions were used chronologically (1) columns never treated with TBABr, using pure THF as solvent, to solubilize samples, and as mobile phase (Section 3.1), (2) THF with various concentrations of TBABr used as solvent and mobile phase (the same lot of solvent prepared to solubilize samples and to use as mobile phase) (Section 3.2) and (3) columns were flushed overnight with THF containing TBABr then used with pure THF as mobile phase (samples were solubilized in pure THF before injection) (Section 3.3). In summary, the samples were always injected in SEC with the same solvent for solubilization and mobile phase, as usual.

3.1. Qualitative description of SEC elution curves

Fig. 1a shows the weight-average molar masses (M_{wi}) as a function of elution volume for a synthetic and a natural poly(*cis*-1,4-

Table 1
Gel rate, number-average molar mass (M_n), weight-average molar mass (M_w), z-average molar mass (M_z) and the z-average radii of gyration (R_{gz}) calculated integrating the all distribution, before treatment of the columns with TBABr[a] (3 replicates/sample).

Sample[b]	Type[c]	Gel rate[d] (%)	M_n (kg/mol)	M_w (kg/mol)	M_z (kg/mol)	R_{gz} (nm)
Natsyn2200	Synthetic PI	24 (2)	550 (10)	1400 (10)	3440 (10)	110 (0.5)
IR305	Synthetic PI	1.5 (0.5)	1070 (20)	2140 (20)	3140 (20)	118 (0.4)
AM	TSR5CV	11.4 (2.5)	570 (50)	1500 (70)	2900 (140)	109 (2.2)
AJ	TSR5	16 (2.9)	810 (30)	1670 (70)	2900 (140)	109 (3.1)
1SAP21	TSR10	35.3 (1.5)	1150 (20)	1640 (20)	2630 (150)	104 (0.4)

[a] TBABr: tetrabutylammonium bromide.
[b] All samples were analyzed as described in Section 2 (two Styragel HMW 6E mixed bed columns, 20 μm, 300 mm × 4.6 mm I.D., flow rate 0.35 mL/min) before TBABr treatment.
[c] PI: poly(*cis*-1,4-isoprene); see Section 2 for specification of NR types.
[d] The value in brackets is the standard deviation.

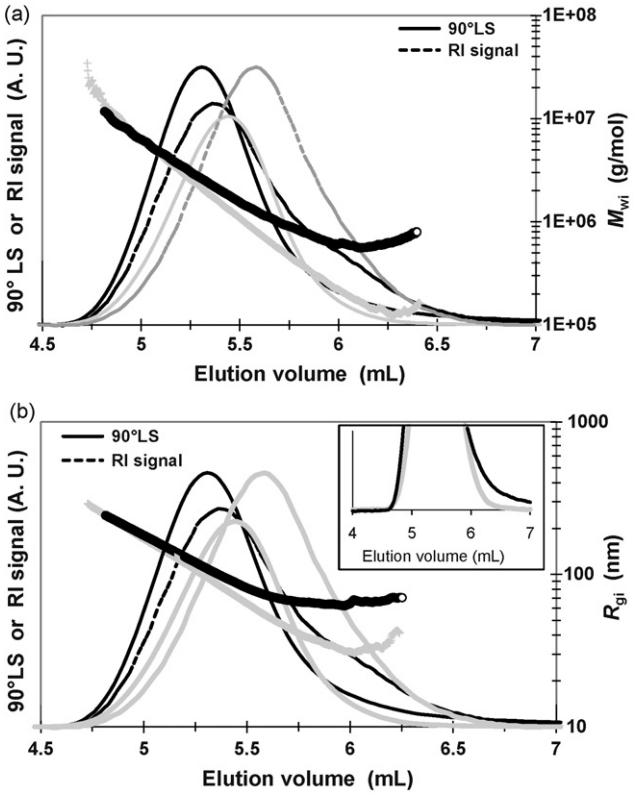

Fig. 1. Chromatograms showing the refractometer (RI) and light scattering (LS, 90°) signals; (a) the molar masses (M_{wi}) and (b) the radius of gyration (R_{gi}) as a function of elution volume of Natsyn2200 (▬) and AJ (▬) samples.

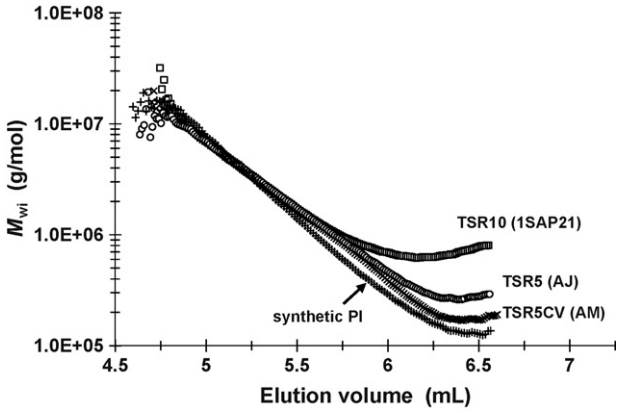

Fig. 2. Plots of weight-average molar masses (M_{wi}) versus elution volume for three NR samples and a synthetic poly(*cis*-1,4-isoprene) (Natsyn2200).

isoprene). For the synthetic poly(*cis*-1,4-isoprene) (Natsyn2200), as expected, M_{wi} decreased linearly as the elution volume increased, up to a point around 5.8 mL. From this elution volume a slight deviation from linearity can be observed. At this elution volume, the signal-to-noise ratio still sufficient cannot be incriminated for the deviation. From an elution volume around 6.2 mL, the signal of the LS detector was too weak and large data fluctuations were found because of a poor signal-to-noise ratio. For the NR sample (1SAP21), M_{wi} as a function of elution volume (V_e) did not follow the same trend as the synthetic poly(*cis*-1,4-isoprene) (SR). The beginning of the elution was similar to SR, decrease of M_{wi} with V_e, but with a lower slope. At a V_e of about 5.5 mL the slope changed and from a V_e in the region of 6.1 mL M_{wi} increased. It appears from these results that fractions of NR with an identical M_{wi} eluted at different elution volumes. The same behaviour was observed for the root mean square radius ($\langle s^2 \rangle^{1/2}$), also known as the radius of gyration (R_{gi}), as a function of elution volume (Fig. 1b). If we observe the baselines of the chromatograms (SR and NR, insert Fig. 1b), the NR baseline from the LS detector did not come back to the initial point, compared to the SR sample. This phenomenon shows that some macromolecules, most probably large entities, were delayed and eluted at a large elution volume where, according to the size-exclusion theory, only small molecules are expected. Fig. 2 shows the plots of molar masses (M_{wi}) versus the elution volume for the three NR samples analyzed (1SAP21 or TSR10, AJ or TSR5 and AM or TSR3CV60, see Section 2 and Table 1 for the difference between types of NR). In fact, the tail varied depending on the NR grade (Fig. 2), particularly depending on the gel rate (Table 1). The less gel there was in the sample (Table 1) involved, the less abnormality there was in the elution profile and, as a result, a lower number-average molar mass (M_n) (Table 1). For the synthetic poly(*cis*-1,4-isoprene), containing about 20% of gel (Table 1), a slight abnormal elution was observed. This anomalous elution behaviour for NR, especially for samples 1SAP21 and AJ, may be due to the fact that large entities are delayed during the elution process. This type of abnormal elution has already been observed mainly for several special polymers (polymacromonomer [13], poly(diphenoxyphosphazene [12], dendrimer [20], or partly crosslink alkyd resins [21]). All these polymers are hyperbranched and/or rigid polymers. Because of the polarity of THF, there is general agreement that the phenomenon is not linked to adsorption between the column packing and part of the polymer, but due to physical interactions (entanglements) between microgel or hyperbranched macromolecules and the column packing according to Podzimek et al. works [22]. Recently, Gaborieau et al. [23] assigned the abnormal elution observed for many polymers to artefacts during data treatment because of a poor signal-to-noise ratio, but not due to branching. It can be seen on Fig. 1 that this reason cannot explain our results because abnormality started for high signal-to-noise ratio.

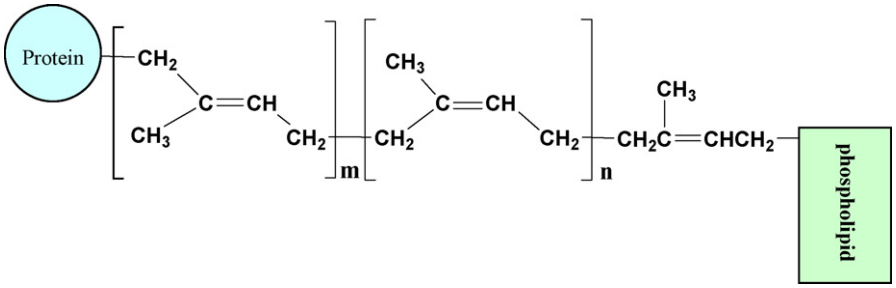

Fig. 3. Structure proposed for poly(*cis*-1,4-isoprene) chains in natural rubber from *Hevea brasiliensis* (see Tarachiwin et al. [8]), links between protein and poly(*cis*-1,4-isoprene) are assumed to be physical bonds, as for links between phospholipids and poly(*cis*-1,4-isoprene) chains.

3.2. Effect of tetrabutylammonium bromide on the elution profiles

Despite the existence of microgel in NR, which might explain the behaviour of NR samples on the basis of Podzimek's hypothesis [22], adsorption cannot be excluded totally for NR. According to the structure of poly(*cis*-1,4-isoprene) chains in NR proposed by Tanaka et al. [3,8] (Fig. 3), it cannot be excluded that the delayed entities for NR was due to adsorption of the polar ends of the chains (protein and phospholipid) on the column packing. Non-size exclusion effects are often mentioned with polar polymers analyzed in the aqueous phase [24,14]. Salts have to be used to avoid delaying some of the macromolecules by adsorption on the column packing [24,9]. To test this hypothesis for NR, tetrabutylammonium bromide was added to THF to solubilize the NR samples and to the THF used as the mobile phase for their analysis by SEC–MALS. TBABr in THF (0.1% or ≈1 g/L) was used by Laguna et al. [25] to analyze poly(diphenoxyphosphazene) by SEC–MALS, but no explanation was given for its use. In our experiments, several concentrations of TBABr (25–1000 mg/L, 0.078–3.1 mM) were tested. Ammonium surfactants can act as catalysts for the oxidation of unsaturated hydrocarbons [26]. Consequently, oxidation of the analyzed natural and synthetic poly(*cis*-1,4-isoprene) was checked before going any further. Fig. 4a shows that the M_w for the synthetic poly(*cis*-1,4-isoprene) samples stayed constant up to a TBABr concentration of 200 mg/L and M_w decreased drastically with more TBABr in the solution. The NR samples did not show any significant oxidation up to a TBABr concentration of 500 mg/L (Fig. 4b). NR sample exhibited more resistance to oxidation compared to SR because of the presence of natural antioxidants in this polymer [27].

For NR sample 1SAP21, the tail quickly reduced under the effect of TBABr, which appeared qualitatively optimum at a concentration of 50 mg/L (Fig. 5). At the optimum concentration of TBABr in the mobile phase (50 mg/L), and for higher concentrations, it was found that the LS signal returned sooner to the initial baseline, in comparison with the NR sample injected without TBABr in the mobile phase (Fig. 6). For the TSR3CV sample (AM), the NR sample with the lower tail (Fig. 2) and gel rate (Table 1), TBABr had no significant action and consequently M_n remained constant, as for synthetic poly(*cis*-1,4-isoprene) (results not shown). In addition, Fig. 7 shows that increasing the concentration of TBABr in THF led to a decrease in gel rate for the two NR samples 1SAP21 and AM, though TBABr had no effect on the gel rate for the synthetic poly(*cis*-1,4-isoprene) Natsyn2200. Adding TBABr to THF made it possible to break down part of the interaction involved in gel formation, but not all, because a plateau was seen from a TBABr concentration of around 50 mg/mL (Fig. 7). As gel in synthetic poly(*cis*-1,4-isoprene) is essentially covalent gel, it is logical that TBABr had no effect on gel rate of Natsyn2200. In NR, it can be imagined that two types of interaction, or links, lie behind gel formation for the samples analyzed. One type of interaction, broken by an ionic surfactant, is probably physical interaction, which tallies with the proposal of Tanaka's team [8]. They suggested the gel was a network which junctions are physical crosslinks between the end-chains (proteins and lipids, Fig. 3). The other type of interaction can be covalent links formed during processing of NR and its storage after processing. Indeed, NR is known to be prone to the so-called storage hardening which generates crosslinks, and gel, by reaction of so-called abnormal groups (carbonyls, amines, epoxy) [17,28]. At this point, it can be noted that the more gel in the NR sample, the more abnormality in SEC elution (tail) and therefore a greater TBABr effect on the elution profile and consequently on M_n.

Plotting R_{gi} versus M_{wi} (conformation plot) for the NR samples treated with 100 mg/L of TBABr (Fig. 8) revealed that both the synthetic poly(*cis*-1,4-isoprene) and the NR sample (1SAP21) analyzed

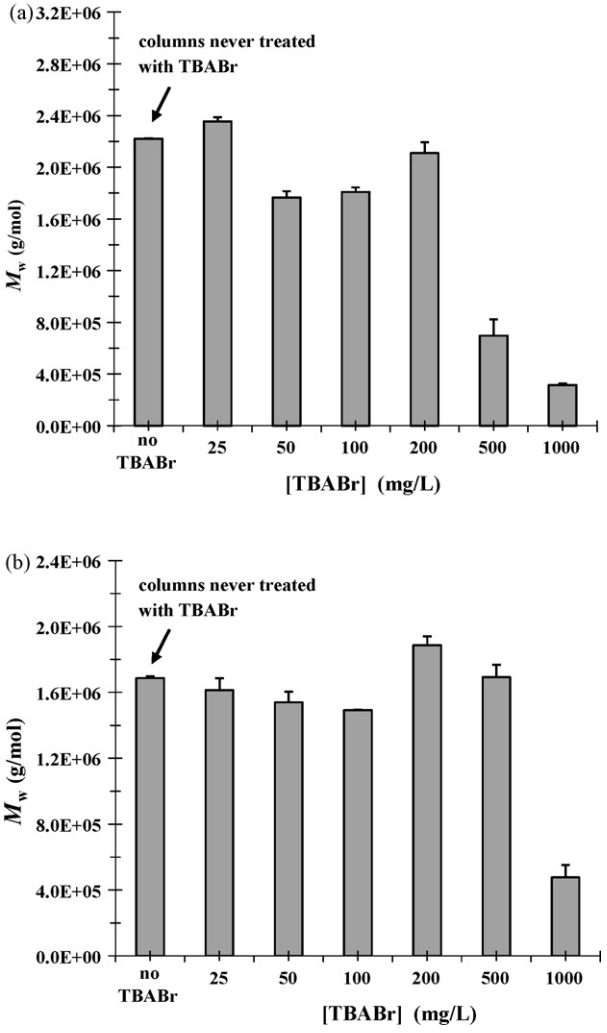

Fig. 4. Influence of TBABr concentration on oxidation for (a) a synthetic PI (IR305, same behaviour as for Natsyn2200, results not shown) and (b) a NR sample (1SAP21, same behaviour as for AM, results not shown) (always same concentration of TBABr in THF used in solvent and mobile phase).

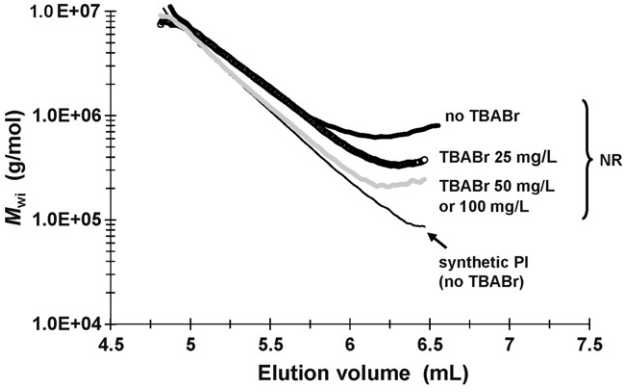

Fig. 5. Logarithmic plots of molar masses (M_{wi}) as a function of elution volume for a synthetic poly(*cis*-1,4-isoprene) (Natsyn2200) and NR sample 1SAP21 analyzed with different concentrations of TBABr in THF.

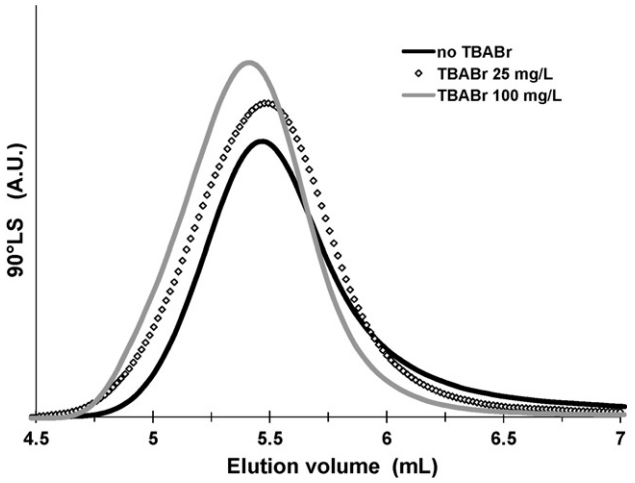

Fig. 6. Chromatograms showing the light scattering (LS, 90°) signal for the NR sample 1SAP21 (no TBABr means the columns were never treated with TBABr).

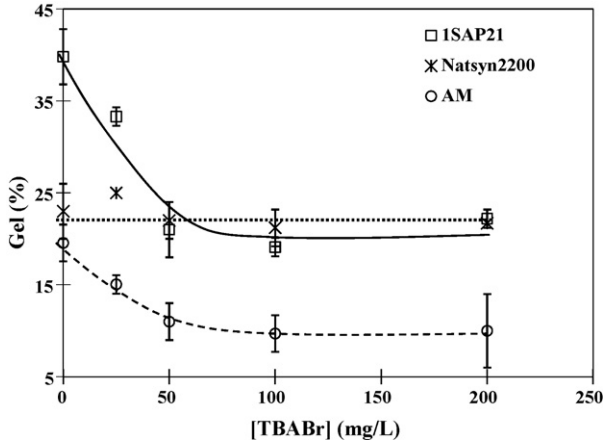

Fig. 7. The effect of TBABr concentration in THF on gel rate for the two NR samples (AM, 1SAP21) and a synthetic sample (Natsyn2200) (curves on the graph are just guides for the eyes used to approximate tendencies).

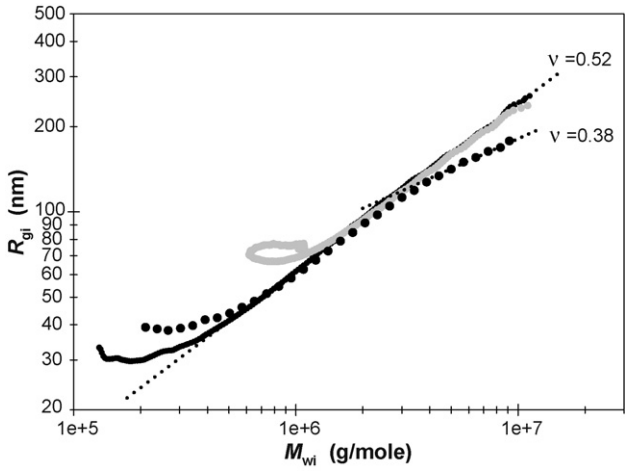

Fig. 8. Log–log plots of R_{gi} versus M_{wi} for the synthetic poly(*cis*-1,4-isoprene) Natsyn2200 (▬) and NR sample 1SAP21 (▬) before TBABr treatment of the columns and NR sample 1SAP21 with 100 mg/mL of TBABr in the mobile phase (●).

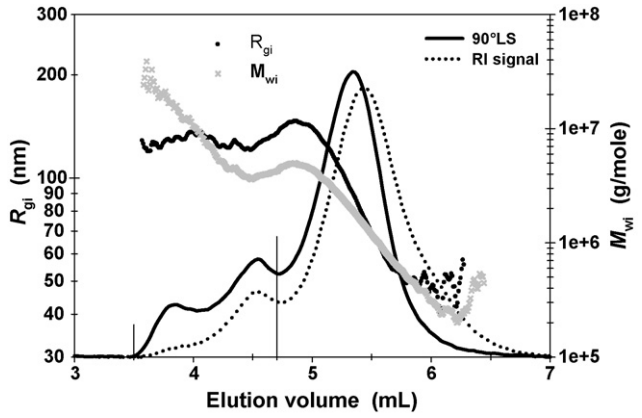

Fig. 9. Chromatograms showing the refractometer (RI) and light scattering (LS, 90°) signals, the molar masses (M_{wi}) and the radius of gyration (R_{gi}) as a function of elution volume for NR sample 1SAP21 injected in pure THF (solvent and mobile phase) after treatment of the columns with THF plus TBABr (3 g/L) 24 h before injection.

with columns never treated with TBABr (solvent and mobile phase being THF) had a Flory exponent (ν) of 0.52. For the NR sample, the upturn for M_{wi} less than 1 million g/mol, corresponding to the abnormality of the elution profile for the higher elution volumes, was not taken into account for calculation of ν. The NR sample (1SAP21) analyzed with 100 mg/L of TBABr in THF no longer had an upturn for 1 million g/mol because of TBABr action as described earlier (Fig. 5). On the other hand, for the higher M_{wi} (2×10^6 to 1×10^7 g/mol), the Flory (ν) exponent decreased drastically to 0.38 (Fig. 8). Two reasons can explain the decrease in ν: either a reduction in the thermodynamic quality of the solvent through TBABr addition, or the presence of branched macromolecules, or compact nano-aggregates (sphere-like). At this stage of the study it can be imagined that the two reasons were involved. Indeed, while this concentration of TBABr (100 mg/L) had no effect on the Flory exponent for both the synthetic poly(*cis*-1,4-isoprene) analyzed (results not shown), large entities such as nano-aggregates can be more sensitive to solvent quality.

3.3. Characterization of the assumed nano-aggregates

The NR samples were injected in SEC in pure THF (solvent and mobile phase), after the treatment of the columns with TBABr. This treatment consisted to flow through the columns a TBABr solution in THF (3 g/L) during 24 h, to be sure to neutralize all polar sites on columns packing. After 24 h, the mobile phase was replaced by pure THF and the system allowed stabilizing for 4 h. Fig. 9 shows injection of the NR sample 1SAP21 in pure THF (solvent and mobile phase), after treatment of the columns with TBABr. Large entities (assumed nano-aggregates) appeared on the chromatogram very early before the elution volume corresponding to normal macromolecules ($V_e \approx 4.7$ mL). The R_g and average molar masses for the assumed nano-aggregate population were calculated from the region defined by the two vertical bars in Fig. 9. These nano-aggregates, eluting between 3.5 and 4.7 mL, amounted to about 10% to 15% of the total material injected in SEC (Table 2), assuming the same dn/dc as poly(*cis*-1,4-isoprene). For the following discussion, the first peak was not considered because of very low RI signal and high variability in the data obtained (Table 2). Considering only the second peak of nano-aggregates (Fig. 9), they appeared to be very compact entities with very large molar masses ($3.6 \times 10^6 < M_n < 4.4 \times 10^6$ g/mol), low polydispersity ($1.02 < I < 1.1$) and a rather low R_g (116–125 nm) given the high M_n (Table 2). Depending on the R_g calculated, it was quite low for the entities

Table 2
Nano-aggregates of three NR samples characterized by SEC–MALS (solvent: pure THF) (3 replicates/sample).

Sample[a]	Type[a]	Quantity[b,c] (% w/w)	M_n (kg/mol)	M_w (kg/mol)	M_z (kg/mol)	I (M_w/M_n)	R_{gz} (nm)
Both peaks calculated together (see Fig. 9)							
AM	TSR5CV	9.9 (0.1)	4 000 (180)	4 800 (320)	7 000 (900)	1.20	114 (1)
AJ	TSR5	14.7 (0.4)	4 550 (110)	5 400 (180)	7 400 (400)	1.18	124 (1)
1SAP21	TSR10	14.9 (1)	4 700 (660)	5 650 (1 000)	7 500 (1 300)	1.20	125 (7)
First peak calculated alone (see Fig. 9)							
AM	TSR5CV	1.4 (0.1)	10 300 (720)	11 650 (1 100)	13 300 (1 800)	1.13	110 (2)
AJ	TSR5	1.5 (0.4)	12 000 (670)	13 100 (740)	14 400 (840)	1.09	124 (2.5)
1SAP21	TSR10	1.3 (0.1)	12 400 (1 600)	13 400 (1 600)	14 600 (1 550)	1.08	128 (5)
Second peak calculated alone (see Fig. 9)							
AM	TSR5CV	8.5 (0.2)	3 600 (160)	3 660 (160)	3 730 (160)	1.02	116 (1)
AJ	TSR5	13.2 (0.4)	4 200 (90)	4 300 (90)	4 450 (100)	1.03	123 (1)
1SAP21	TSR10	13.6 (1)	4 400 (650)	4 850 (1 000)	5 550 (1 650)	1.1	125 (9)

[a] See Section 2 for specification of NR types.
[b] %, w/w of material injected in SEC, based on dn/dc = 0.130 mL/g.
[c] The value in brackets is the standard deviation.

eluting so early ($3.5 < V_e < 4.7$ mL). Indeed, poly(*cis*-1,4-isoprene) chains with a R_g value of about 115 nm eluted at 5.3 mL. The hydrodynamic volume of those entities was therefore rather higher than the poly(*cis*-1,4-isoprene) chains. These results, a low R_g and a high apparent hydrodynamic volume, suggest that these entities were not random coil polymers but more probably sphere-like entities. Indeed, micelles [29] or microgels [30] are characterized by ratios R_h/R_g (R_h for hydrodynamic radius) higher than for real random coil ($R_h/R_g = 0.64$ [10]). Kai Wu et al. [29] found this ratio for nano-micelles ($15 < R_g < 25$ nm) varying between 1.6 and 1.8. Quite the same values were given by Boyko et al. [30] for microgels whose R_g were about 150–225 nm. As previously mentioned, Tarachiwin et al. [8] suggested polyisoprene chains in NR had the two end chains linked one to a peptide (or protein) and the other one to a phospholipid (or a fatty acid) (Fig. 3). This structure has a certain homology with the zwitterionic end ω-functionalized monodisperse polyisoprenes studied by Hadjichristidis et al. [31]. These types of polymers, terminated at one end by a dipolar group (Fig. 10) had the property to form micelles especially in cyclohexane. This property was very dependant of the molar mass of the zwitterionic homopolymer: the lower the molar mass the higher the degree of association to form micelles. The degree of association depends also on the polarity of the end groups and the solvent. In addition, Davidson et al. [32] showed that the addition of 1–5% of n-heptanol in cyclohexane reduced greatly aggregation rate of zwitterionic polyisoprene. The same phenomenon was observed for natural rubber, adding 10% of ethanol in cyclohexane decreased significantly the gel rate (from 46% to 15%) [33]. On the basis of these results and assuming more polar end groups for NR, it can be postulated that in the case of NR, the short chains are involved in the formation of nano-micelles. One other possibility would be the presence of hydrophobic protein aggregates in the nano-aggregates. Additional experiments are necessary to go further for elucidation of the structure and composition of these nano-aggregates.

It was very important to treat the columns with THF plus TBABr just before the analysis. Indeed, over time the peaks corresponding to the nano-aggregates tended to decrease and to elute at a higher elution volume (Fig. 11). This phenomenon means that TBABr was washed out of the columns over time when using pure THF and adsorption on the column packing increased. This result confirms that, for NR samples, the main factor delaying nano-aggregates is most probably adsorption on the column packing.

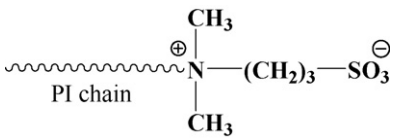

Fig. 10. Example of the structure of an end group for zwitterionic end group ω-functionalized polyisoprene (PI) studied by Hadjichristidis et al. [31].

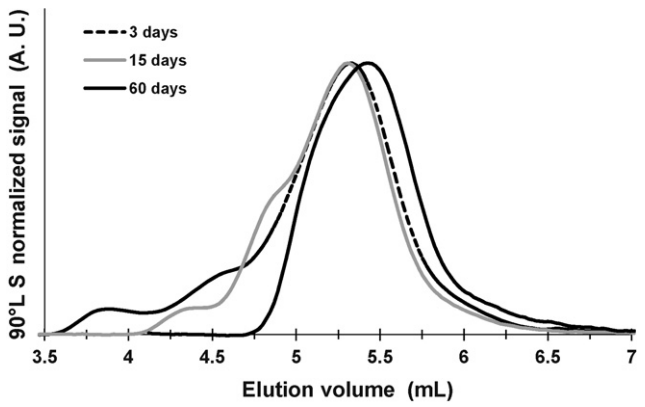

Fig. 11. Chromatograms showing the light scattering (LS, 90°) signals as a function of elution volume for NR sample 1SAP21 injected in pure THF (solvent and mobile phase) 3, 15 and 60 days after treatment of the columns with THF plus TBABr (3 g/L).

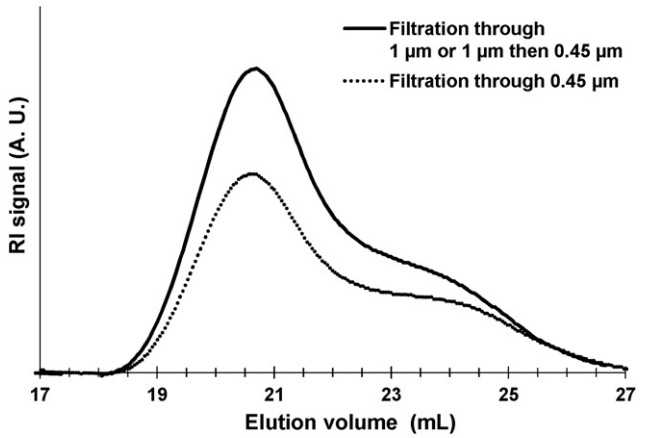

Fig. 12. Chromatograms showing the refractometer signal (RI) as a function of elution volume of a NR solution (AN) after filtrations through 0.45 µm, 1 µm and double filtration through 1 µm then 0.45 µm.

Table 3
Effect of filtration on apparent gel rates and average molar masses of natural and synthetic poly(*cis*-1,4-isoprene) samples after filtrations through filters of different porosities[a].

Sample[b]	Type[b]	Gel rate[c,d] (% w/w)			M_n (kg/mol)			M_w (kg/mol)		
		Filters			Filters			Filters		
		0.45 μm	1 μm	1 μm + 0.45 μm	0.45 μm	1 μm	1 μm + 0.45 μm	0.45 μm	1 μm	1 μm + 0.45 μm
CB	TSR5CV	47 (4)	28 (1)	29 (1)	540 (40)	620 (10)	630 (3)	1250 (50)	1360 (20)	1340 (20)
AN	TSR5	54 (3)	32 (1)	34 (3)	600 (50)	850 (40)	810 (30)	1260 (50)	1490 (50)	1460 (50)
1SAP21	TSR10	73 (3)	54 (2)	54 (2)	520 (50)	810 (70)	880 (20)	1010 (50)	1250 (60)	1310 (90)
Natsyn2200	PI	79 (6)	24 (2)	26 (1)	300 (20)	460 (50)	450 (10)	860 (100)	1000 (120)	1090 (10)
IR305	PI	3 (2)	1 (1)	2 (2)	1050 (20)	1080 (70)	1050 (60)	2220 (30)	2180 (30)	2150 (20)

[a] PLgel mixed-A 300 mm × 7.5 mm I.D., 20 μm, Polymer Laboratories, Amherst, MA, USA, at a flow rate of 0.65 mL/min in THF.
[b] See Section 2 for specification of NR types.
[c] %, w/w of material injected in SEC.
[d] The value in brackets is the standard deviation.

3.4. Influence of filter porosity

Filtering NR solutions through 0.45 μm filters (PTFE membrane) gave chromatograms changed, lower average molar masses and higher apparent gel rates compared to results obtained after filtration through 1 μm filters (glass fibre membrane) (Fig. 12) (Table 3). Three NR samples of different grades were tested. The sample BC (TSR5CV) had a gel rate increasing from 28% (1 μm) to 47% (0.45 μm). The sample AN (TSR5) had a gel rate increasing from 32% (1 μm) to 54% (0.45 μm). And the sample 1SAP21 (TSR10) had a gel rate increasing from 54% (1 μm) to 73% (0.45 μm). As in the case of NR samples, the gel rate of Natsyn2200 also increased, from 24% to 79%, and the average molar masses decreased after filtration through 0.45 μm compared to 1 μm. IR035 had a negligible gel rate at 1 μm. Filtering IR305 solution either through 1 μm or 0.45 μm gave the same results for gel rates and average molar masses. For all samples (Table 3), filtering first through 1 μm then 0.45 μm (double filtration) gave the same results as filtration through 1 μm only. Absent of gel for the sample IR305, there was no blockage and hence no retention of macromolecules when the 0.45 μm was used. Higher increase of apparent gel rate of Natsyn2200 compared to NR samples explains the blocking of the pore by gels was more or less rapid depending on the nature of gels. For NR samples and Natsyn2200, the gels progressively reduced the porosity of the 0.45 μm filter, resulted in an artefact increasing of apparent gel rates and decreasing of average molar masses due to retention of macromolecules, especially high molar masses. Bonfils and Char [7] in a previous study on this subject misinterpreted this phenomenon by proposing an over-shearing of the polyisoprene chains on the membrane of the filter. Filtering solutions through 1 μm filter could remove only gel and did not cause the retention of macromolecules. It is therefore important to filter solutions of natural rubber through filters of porosity of 1 μm instead of, or before to filter through 0.45 μm porosity.

4. Conclusion

The study of the filter porosity revealed the importance of filtering the solutions of rubber either on 1 μm porosity or on 1 μm then 0.45 μm to avoid blocking of the 0.45 μm filters and artefacts. The gel rate will be overestimated and the average molar masses underestimated.

SEC–MALS characterization of NR samples showed an abnormal elution profile at high elution volumes, with the phenomenon depending on the gel rate in the NR sample. Compared to a synthetic poly(*cis*-1,4-isoprene), after regular elution at small elution volumes, the molar mass of the eluting natural polyisoprene increased with an increasing elution volume. By this phenomenon, molar masses of NR measured by SEC–MALS must be used with caution. The addition of different concentrations of TBABr in THF led to a reduction in elution volume of large entities responsible for abnormal elution; and a decrease in gel rate probably by breaking up interactions between the poly(*cis*-1,4-isoprene) chains. An analysis of NR samples in pure THF (solvent and mobile phase) after treatment of the columns with a solution of TBABr in THF (3 g/L) made it possible to separate the large entities from the poly(*cis*-1,4-isoprene) chains, with the former eluting earlier. These results argue in favour of adsorption of the large entities on the column packing.

These large entities are assumed to be nano-aggregates having a very compact structure comparable to a sphere, probably nano-gels or nano-micelles. At this stage of our research, it is not very clear why the nano-aggregates are visible, with columns treated with TBABr, in pure THF as mobile phase but not if the mobile phase contains TBABr. Knowing composition of NR, it can be thought that proteins are involved in the nano-aggregates. Studies are in progress in our laboratory to understand these phenomena and for a more complete characterization of these nano-aggregates.

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