European Polymer Journal 45 (2009) 2249–2259

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Study of chain branching in natural rubber using size-exclusion chromatography coupled with a multi-angle light scattering detector (SEC-MALS)

Chandy Kim [a], Jerome Sainte Beuve [b], Stephane Guilbert [c], Frédéric Bonfils [b,*]

[a] Cambodian Rubber Research Institute, P.O. Box 1337, Phnom Penh, Cambodia
[b] UMR 1208 Ingénierie des Agropolymères et Technologies Emergentes, CIRAD, INRA, Montpellier SupAgro, Université de Montpellier 2, UMR IATE – TA B 62/16, 73, rue JF Breton, 34398 Montpellier Cedex 5, France
[c] UMR 1208 Ingénierie des Agropolymères et Technologies Emergentes, CIRAD, INRA, Montpellier SupAgro, Université de Montpellier 2, UMR IATE – Bât. 35, 2, place Viala, F-34000 Montpellier, France

ARTICLE INFO

Article history:
Received 19 March 2009
Received in revised form 6 May 2009
Accepted 10 May 2009
Available online 18 May 2009

Keywords:
Natural rubber
Branching
Nanoaggregate
SEC-MALS

ABSTRACT

The different rheological behaviour of natural rubber (NR) compared to industrial synthetic poly(*cis*-1,4-isoprene) (SR) has been attributed to the gel phase and long-chain branching. Previous studies on branching in NR were carried out using the fractionation technique by precipitation to obtain narrow molar mass distribution. In this study, chain branching of poly(*cis*-1,4-isoprene) in NR was characterised by size-exclusion chromatography coupled with an online multi-angle light scattering detector (SEC-MALS). The nanoaggregates adsorbed on the column packing interfered with branching characterisation for short and medium chains (M_w < 1000 kg/mol). Using a master curve of linear standard poly(*cis*-1,4-isoprenes), SEC-MALS revealed no or very little branching in the higher chains (1000 < M_w < 10,000 kg/mol) of natural rubber contrary to previous studies. This study showed that the soluble portion of NR samples was composed of almost linear poly(*cis*-1,4-isoprene) and nanoaggregates with rather compact structures.

1. Introduction

The rheological properties of natural rubber from *Hevea brasiliensis* (NR) are very different from those of synthetic poly(*cis*-1,4-isoprene) and even Guayule rubber [1,2]. In particular, NR exhibits greater viscosity and a lower relaxation rate. These specific properties have been attributed to the presence of a gel phase, an insoluble fraction in good solvents for poly(*cis*-1,4-isoprene) [3–5], along with long-chain branching on the macromolecules [6]. These long-chain branching in NR were studied after fractionation by successive precipitations [6–8]. The fractions of different molar mass were characterised either by coupling size-exclusion chromatography (SEC) and viscometry measurements (intrinsic viscosity, [η]) [6,7], or combining NMR and osmometry [8]. Using Zimm and Kilb's relations [9] they determined the number of branching points per chain (m), assuming either tri-functional or tetra-functional branching points. The values of m varied with weight-average molar mass (M_w) and NR purification. Angulo-Sanchez and Caballero-Mata [7] showed that m varied from 2.4 (M_w = 361 kg/mol) to 4.9 (M_w = 2450 kg/mol) in non-purified NR, assuming tetra-functional branching points. Fuller and Fulton [6] found m ranging from 0.6 (M_w = 420 kg/mol) to 6.4 (M_w = 2240 kg/mol). Sakdapipanich et al. [8] worked on purified NR (deproteination), and found much lower m, from 0.3 to 1.3, assuming tetra-functionalities. These values decreased to zero after additional purification by transesterification of the rubber.

Today, to avoid the tedious fractionation steps necessary to obtain polymers with narrow molar mass distribution and structural modifications throughout the different fractionation stages, size exclusion chromatography

* Corresponding author. Tel.: +33 467614430; fax: +33 467616547.
E-mail address: frederic.bonfils@cirad.fr (F. Bonfils).

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doi:10.1016/j.eurpolymj.2009.05.015

Table 1
SEC-MALS analyses of standard linear PS using the Zimm and Berry fit methods (mean values of two replicates).

Standard sample	M_w (kg/mol)[a]						I_p (M_w/M_n)		R_g (nm)[c]		
	[b]	Zimm		Berry		[d]	Zimm	Berry	Zimm	Berry	[d]
PS1	30	28.7	4.3%	28.7	4.3%	ns	1.00	1.00	–	–	–
PS2	120	119.8	0.2%	120.2	0.2%	ns	1.00	1.00	12.2	15.4	ns
PS3	200	203.8	−1.9%	204.0	−2%	ns	1.01	1.01	17.1	17.7	ns
PS4	460	416.8	9.4%	416.9	9.4%	ns	1.02	1.02	27.1	27.3	ns
PS5	1460	1384	5.2%	1369	6.2%	ns	1.01	1.01	58.7	55.6	ns
PS6	2650	2944	−11.1%	2835	−7%	ns	1.05	1.04	98.3	88.6	s
PS7	7100	6900	2.8%	6371	10.3%	s	1.10	1.06	170.3	144.7	s

[a] Weight-average molar mass obtained using the Zimm and Berry fit methods, coefficient of variation less than 3%, the values in percentage were the differences between values obtained from our experiments and those provided by the supplier.
[b] Weight-average molar mass provided by the supplier.
[c] *z*-Average radii of gyration obtained using the Zimm and Berry fit methods.
[d] s or ns: significant or not significant difference between fit methods (at $P < 0.05$).

coupled with multi-angle light scattering (SEC-MALS) is used for direct branching characterisation of polymers [10,11]. SEC-MALS is a very versatile technique since the light scattering detector provides absolute M_w and 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) and the distribution of branching depending on size or molar mass. The only limitation of this method is having a polydisperse linear reference to compare the supposed branched polymers. Usually, a less branched polymer is chosen from a set of polymers analysed. One alternative might be to use a master curve, or model plot, $R_g = f(M_w)$, obtained from a set of linear polymer standards with the same chemical structure. This method would have the advantage of determining an absolute number of branchings per chain and enable a better comparison with data in the literature. Recently, Kim et al. [12] showed that NR samples analysed by SEC-MALS gave abnormal elution at high elution volume, probably because of highly compact nanoaggregates or nanomicelles delayed by interactions with the column packing.

This paper deals with the strategy used to characterise the absolute number of branching per chain in samples of non-purified (raw) natural rubber by SEC-MALS.

2. Experimental

2.1. Materials

The NR samples used for this study were all TSRs (Technically Specified Rubber). They were prepared in a rubber processing factory in Cambodia according to usual TSR processing (acid, or natural coagulation of field latex, crumbing, washing and drying). Sample 1SAP21 was a TSR10 grade prepared by natural coagulation of latex followed by 4 days of coagulum maturation before processing. Sample AN was prepared by acid coagulation (formic acid) of fresh field latex, it was a TSR5 grade. Samples AW, AG, AB and AC 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 [13]. Three synthetic poly(*cis*-1,4-isoprenes), Natsyn2200 (Goodyear chemical), IR305 (Kraton polymers) and Nipol2200 (Zeon Corporation), were used directly as received. Standard linear poly(*cis*-1,4-isoprene) (PI) and standard linear polystyrene (PS) were obtained from Polymer Standard Service (PSS, Germany) and were used as received.

Table 2
SEC-MALS analyses of standard linear PI using the Zimm and Berry fit methods (mean values of 3 replicates).

Standard sample	M_w[a] (kg/mol)						I_p (M_w/M_n)		R_g[c] (nm)		
	[b]	Zimm		Berry		[d]	Zimm	Berry	Zimm	Berry	[d]
PI1	110	107.3	2.4%	107.4	2.4%	ns	1.00	1.01	14.5	15.3	ns
PI2	157	152.6	2.8%	152.8	4.2%	ns	1.01	1.01	18.1	18.7	ns
PI3	314	279.4	11%	278.7	11.2%	ns	1.05	1.05	27.1	26.7	ns
PI4	436	385.8	11.5	385.5	11.6%	ns	1.01	1.01	32.9	32.6	ns
PI5	576	505.2	12.3%	503.1	12.6%	ns	1.06	1.06	40.2	38.9	ns
PI6	735	668.2	9.1%	663.3	9.7%	ns	1.06	1.06	48.2	46.3	ns
PI7	1000	928.6	7.1%	915.5	8.4%	ns	1.02	1.02	58.9	55.5	ns

[a] Weight-average molar mass obtained using the Zimm and Berry fit methods, coefficient of variation less than 1.5%, the values in percentage were the differences between values obtained from our experiments and those provided by the supplier.
[b] Weight-average molar mass provided by the supplier.
[c] *z*-Average radii of gyration obtained using the Zimm and Berry fit methods.
[d] s or ns: significant or not significant difference between fit methods (at $P < 0.05$).

2.2. Size-exclusion chromatography with light scattering detector

The samples (25 ± 5 mg) were dissolved in tetrahydrofuran (THF, 40 ml, HPLC grade) stabilized with 2,6-di-*tert*-butyl-4-methylphenol (BHT). After storing 14 days in the dark at 30 °C, the solutions were filtered (1 μm, glass fibre, Pall) and injected in SEC-MALS. As the exact initial concentration of the sample solutions was known and the injected quantity was determined after filtration and elution, it was 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 three PLgel (Polymer Laboratories) Mixed-A mixed bed columns (20 μm, 300 mm × 7.8 mm I.D.) with a guard column. The columns were maintained at 45 °C. The mobile phase was THF at a flow rate of 0.65 ml/min; the injected volume was 150 μl.

For experiments done to evaluate the effect of tetrabutylammonium bromide (TBABr) on delayed nanoaggregates, the columns were flushed overnight with THF containing TBABr (3 g/L) prior to SEC analyses. Afterwards, the mobile phase was changed back to pure THF for analysis of NR samples.

2.3. Data analysis for MALS detectors and short theoretical background

The data obtained with MALS detectors were analysed with ASTRA software (version 5.3.2.22) (Wyatt Technology, Santa Barbara, CA) using a fit method, usually the Berry fit method or the Zimm method. Of course, all the calculations in Astra were performed on the assumption that particular elution volume slices were monodisperse or very narrow. For the Zimm fit method $Kc/\Delta R(\theta)$ was plotted against $\sin^2(\theta/2)$, Eq. (1), while for the Berry fit method the square root of $Kc/\Delta R(\theta)$ was plotted against $\sin^2(\theta/2)$, Eq. (2). The 1st order polynomial fit was used in the Zimm fit method for the calculations of M_w and R_g, but a 2nd order polynomial fit was used in the Berry fit method. The angles no. 2, 17 and 18 were excluded from the calculation, for all samples and fitting methods, because of erroneous fitting.

$$\frac{Kc}{\Delta R(\theta)_i} = \frac{1}{M_{wi}} + \frac{16\pi^2 n_0^2}{3\lambda_0^2}\frac{\langle R_g^2\rangle_i}{M_{wi}}\sin^2(\theta/2) \quad (1)$$

$$\left[\frac{Kc}{\Delta R(\theta)}\right]_i^{1/2} = \left[\frac{1}{M_{wi}} + \frac{16\pi^2 n_0^2}{3\lambda_0^2}\frac{\langle R_g^2\rangle_i}{M_{wi}}\sin^2(\theta/2)\right]^{1/2} \quad (2)$$

where, $\Delta R(\theta)$ is the excess Rayleigh ratio, the ratio of scattered and incident light intensity; c is the solute concentration in g/ml, K is an optical constant, Eq. (3), θ is the scattering angle, M_{wi} and R_{gi} are, respectively, the weight-average molar mass and z-average radius of gyration for elution slice i.

$$K = \frac{4\pi^2 n_0^2}{N_A \lambda_0^2}(dn/dc)^2 \quad (3)$$

n_0 is the refractive index of the solvent; N_A is Avogadro's number; λ_0 is the wavelength of the laser beam in a

Table 3
SEC-MALS analyses of industrial synthetic poly(*cis*-1,4-isoprene) and natural rubber samples using the Zimm and Berry fit methods.

Sample	Grade	Clone	M_w[a] (kg/mol)				I_p (M_w/M_n)			$R_{g,z}$[b] (nm)				ν[c]		
			Zimm	$Zimm_{3-8}$	Berry	d	Zimm	$Zimm_{3-8}$	Berry	Zimm	$Zimm_{3-8}$	Berry		Zimm	$Zimm_{3-8}$	Berry
IR305	PI		2270	2210	2170	ns	2.16	2.15	2.07	134	127	115	s	0.61	0.61	0.55
Natsyn2200	PI		1180	1150	1140	ns	2.66	2.53	2.52	111	104	95	s	0.59	0.57	0.53
Nipol2200	PI		1680	1640	1590	ns	2.52	2.34	2.31	139	130	113	s	0.59	0.56	0.52
1SAP21	TSR10	PR107	1350	1360	1340	ns	1.58	1.48	1.48	106	101	94	s	0.61	0.60	0.56
AN	TSR5	PR107	1550	1520	1490	ns	1.88	1.76	1.75	121	115	104	s	0.62	0.61	0.56
AB	TSR5CV	PB217	1600	1570	1540	ns	2.25	2.11	2.09	125	118	106	s	0.60	0.60	0.55
AW	TSR5CV	PR107	1440	1410	1380	ns	2.90	2.68	2.65	124	118	106	s	0.60	0.59	0.56
AG	TSR5CV	RRIM600	1500	1470	1430	ns	3.01	2.74	2.74	129	122	109	s	0.59	0.61	0.55

[a] Weight-average molar mass obtained using the Zimm, Zimm with angles 3–8 and Berry fit methods.
[b] z-Average radii of gyration obtained using the Zimm, Zimm with angles 3–8 and Berry fit methods.
[c] Flory exponent obtained using the Zimm, Zimm with angles 3–8 and Berry fit methods.
[d] s or ns: significant or not significantly different between the Zimm and Berry fit methods ($P < 0.05$).

vacuum (633 nm for Dawn DSP, Wyatt technology); and d*n*/d*c* is the differential refractive index increment of the polymer in the solvent used. For SR and NR the d*n*/d*c* at 633 nm determined previously was 0.130 mg/l [12].

2.4. Estimation of the number of branchings per chain

SEC-MALS provides M_{wi} and R_{gi} for every slice of the chromatogram. The combination of M_{wi} and a size parameter, R_{gi}, in the case of polydisperse polymers, is used to obtain information about branching characteristics. The branching characteristics of a polymer can be evaluated by the branching index [14,11] (also called contraction factor [15], Eq. (4)). At the same molar mass, R_g of a branched chain is smaller than that of a linear chain.

$$g = \frac{R_{g^2,b}}{R_{g^2,l}} \tag{4}$$

The subscripts *b* and *l* represent the branched and linear chains, respectively.

The number of branching points per chain of polymer, m_3 (tri-functional) and m_4 (tetra-functional), can be calculated using the Zimm and Stockmayer relation [15]. The relationships between *g* and m_3 or m_4 were given in Eqs. (5) and (6).

$$g = \left[(1 + m_3/7)^{1/2} + 4m_3/9\pi\right]^{-1/2} \tag{5}$$

$$g = \left[(1 + m_4/6)^{1/2} + 4m_4/3\pi\right]^{-1/2} \tag{6}$$

3. Results and discussion

3.1. Influence of the fit method

For analyses of the data obtained with MALS detectors, the Zimm or Berry fit methods can be used with Astra software. These methods are different in terms of the mathematical process used for data extrapolation (see Section 2). Anderson et al. [16] showed that the Zimm method led to overestimation of R_g above 50 nm. The Berry fit method was shown to be better for analysing high molar mass PEO [16]. Therefore, in this study on natural rubber (NR) samples, the Zimm and Berry fit methods were compared for a set of standard linear polystyrenes (PS) (Table 1) and of standard linear poly(*cis*-1,4-isoprenes) (PI) (Table 2). For the standard linear PS, the M_w obtained using both fit methods were not significantly different, except PS7, but the R_g were significantly different for PS6 and PS7 (Table 1). The calculated value of R_g was more angular-dependent than the one of M_w and was thus more affected by the fit method used. From the data displayed in Table 1, the conformation plot, $R_g = f(M_w)$, gave $R_g = 0.0059 M_w^{0.652}$ for the Zimm fit method (PS2–PS7) and $R_g = 0.0101 M_w^{0.611}$ for the Berry fit method (PS3–PS7). The equation of the conformation plot obtained using the Berry fit method tallied quite well with Nakamura et al. [17] and Terao and Mays [18] who found $R_g = 0.0118\ M_w^{0.6}$, but without any indication of the fit method used. Table 2 compares structural parameters for standard linear PI obtained with SEC-MALS using the Zimm and Berry fit methods. It was not possible to find commercial standard linear PI with M_w higher than 1000 kg/mol, corresponding to R_g of about 55–60 nm (Table 2). There was no significant difference between fit methods for all PI standards tested (Table 2). The results obtained with SEC-MALS were significantly lower than the values given by the supplier. The equations of the conformation plot of the standard linear PI obtained using the Zimm and Berry fit methods were $R_g = 0.0074\ M_w^{0.654}$ and $R_g = 0.0135\ M_w^{0.606}$, respectively. The Flory exponents of the standard linear PI obtained using the Zimm and Berry fit methods were 0.654 and 0.606, respectively.

The influence of the fit method on the analyses of industrial polydisperse synthetic poly(*cis*-1,4-isoprene) (SR) and natural rubber (NR) was also checked. All industrial SR had the same M_w obtained using both fit methods, but their R_g were significantly different (Table 3). The Zimm fit method gave higher values for the Flory exponent than the Berry fit method. For sample IR305, different equations of the conformation plot with *v* equal to 0.61 (Zimm fit method) and 0.55 (Berry fit method) were obtained. For sample Natsyn2200 the Flory exponents were 0.59 for the Zimm fit method and 0.53 for the Berry fit method. Sample Nipol2200 had the largest difference in Flory exponent values between the two methods: 0.59 for the Zimm fit method and 0.52 for the Berry fit method (Table 3). For NR samples, M_w values determined using the Zimm and Berry fit methods were not significantly different. However, the R_g were significantly different between the two fit methods, as

Table 4
Contraction factor (*g*) and number of branched points per chain (m_4) of industrial synthetic poly(*cis*-1,4-isoprene) and natural rubber samples at M_{wi} 6000 kg/mol obtained with SEC-MALS using Berry fit method.

Sample	Grade	Clone	Number of analyses over 1 year[a]	Gel (%)	*v*[b]	*g* (6000 kg/mol)[c]	m_4 (6000 kg/mol)[c]
IR305	PI		15	0	0.553 ± 0.005	0.89 ± 0.01	0.26 ± 0.02
Natsyn2200	PI		12	24	0.529 ± 0.004	0.77 ± 0.01	0.70 ± 0.05
Nipol2200	PI		6	11	0.518 ± 0.005	0.75 ± 0.05	0.78 ± 0.05
1SAP21	TSR10	PR107	12	49	0.556 ± 0.015	0.81 ± 0.06	0.56 ± 0.19
AN	TSR5	PR107	12	30	0.559 ± 0.006	0.88 ± 0.02	0.27 ± 0.06
AB	TSR5CV	PB217	9	17	0.552 ± 0.009	0.84 ± 0.05	0.50 ± 0.10
AW	TSR5CV	PR107	12	22	0.557 ± 0.006	0.87 ± 0.02	0.32 ± 0.06
AG	TSR5CV	RRIM600	3	17	0.553 ± 0.014	0.84 ± 0.01	0.44 ± 0.04

[a] Each solution was prepared according experimental section and injected once after 14 days.
[b] Flory exponent with 95% confidence interval.
[c] With a 95% confidence interval.

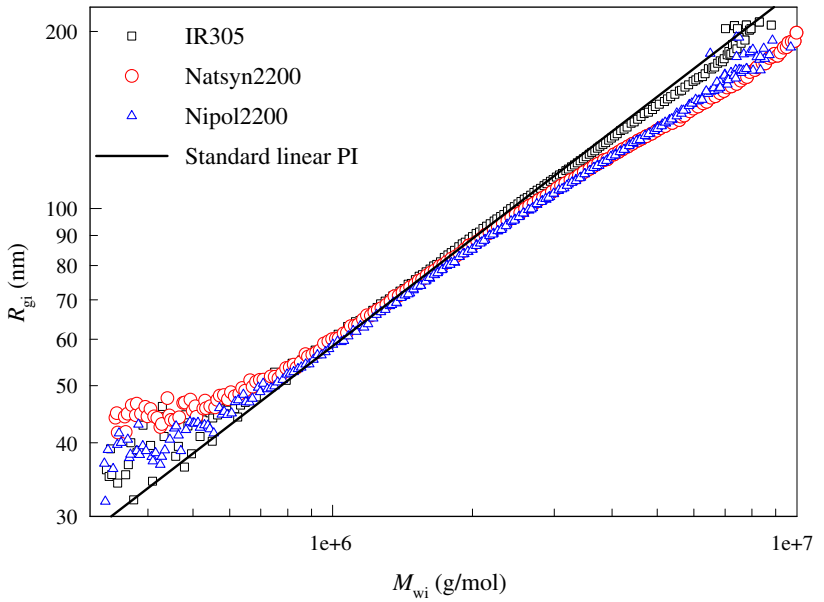

Fig. 1. Conformation plots of industrial synthetic poly(*cis*-1,4-isoprene) samples obtained with SEC-MALS (the straight line is the conformation plot of the standard linear PI).

observed in the case of SR samples (Table 3). When using the Zimm fit method with low angles (3–8), the results obtained were not significantly changed, although the difference with the Berry fit method was minimised. The NR samples had Flory exponent values ranging from 0.60 to 0.62 for the Zimm fit method and from 0.55 to 0.56 for the Berry fit method (Table 3).

The Flory exponents of the standard linear PI and standard linear PS obtained using the Zimm fit method (Tables 1 and 2) appeared rather overestimated. Indeed, in theory, ν for a random coil polymer in a good solvent is a maximum of 0.6 [15]. For the SR and NR samples, the Zimm fit method also gave overestimated values for the Flory exponent. Based on the results obtained, the Berry fit

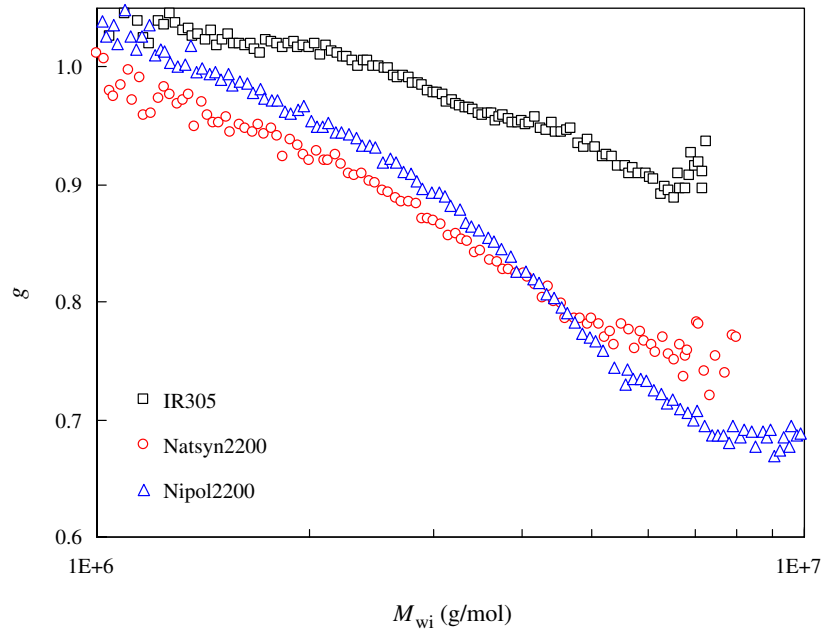

Fig. 2. Distributions of the contraction factor, g, of industrial synthetic poly(*cis*-1,4-isoprene) samples as a function of molar mass using linear standard PI as a reference.

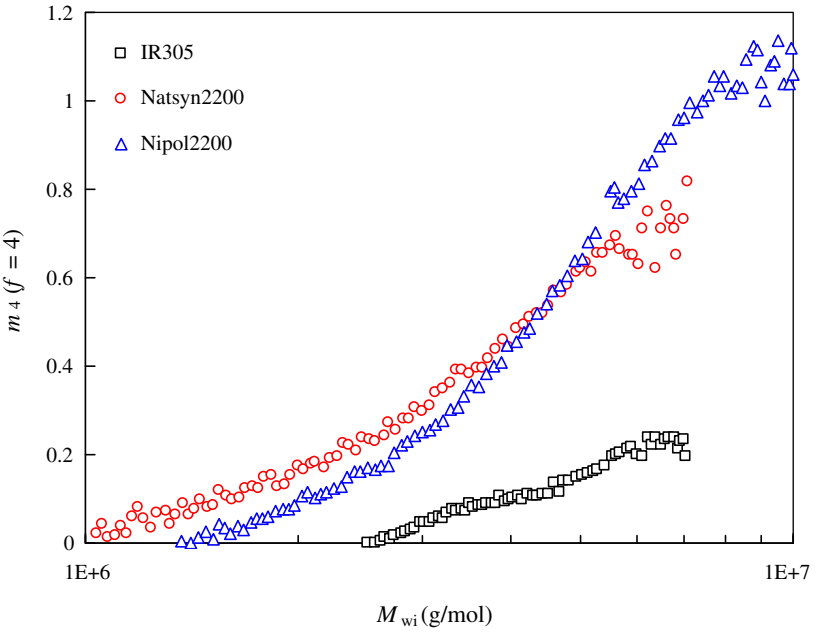

Fig. 3. Distributions of the number of branching per chain (m_4) of industrial synthetic poly(*cis*-1,4-isoprene) samples as a function of molar mass using linear standard PI as a reference.

method appeared to be a better method than the Zimm fit method for studying branching in NR.

3.2. Branching analysis using SEC-MALS

A reference polydisperse polyisoprene with linear chains, or a master curve obtained with standard linear PI, is needed to calculate the number of branching points per chain (m) using SEC-MALS and Astra Software. In order to determine the more absolute branching parameters, especially for comparison with data in the literature, we decided to compare the samples analysed to the master curve obtained with standard linear polyisoprene after Berry fit calculation ($R_g = 0.0135 M_w^{0.606}$) (Table 2). Samples IR305 (ν = 0.553, Table 4), Natsyn2200 (ν = 0.529, Table 4) and Nipol2200 (ν = 0.518, Table 4) displayed conformation plots with gentler slopes compared to that for the standard linear PI (solid strait line) (Fig. 1). The lower ν values

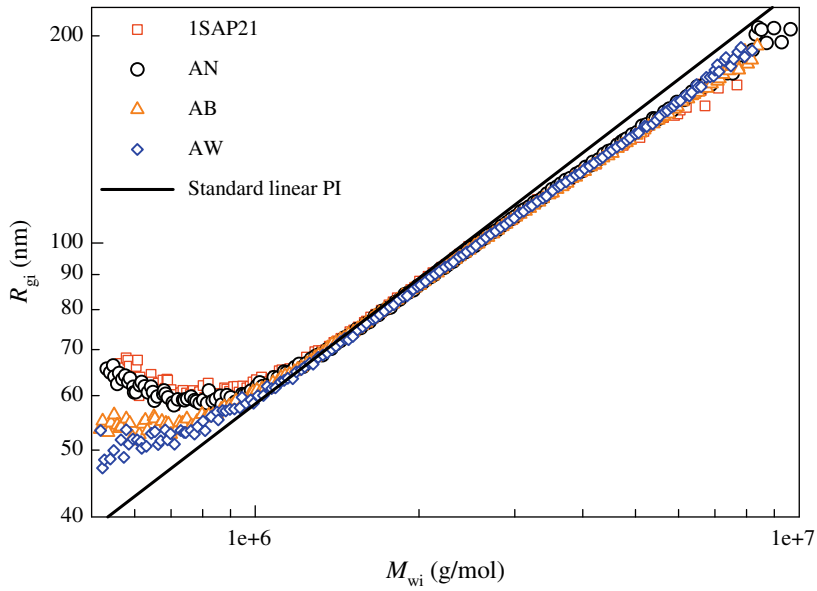

Fig. 4. Conformation plots of NR samples obtained with SEC-MALS (the straight line is the conformation plot of the standard linear PI).

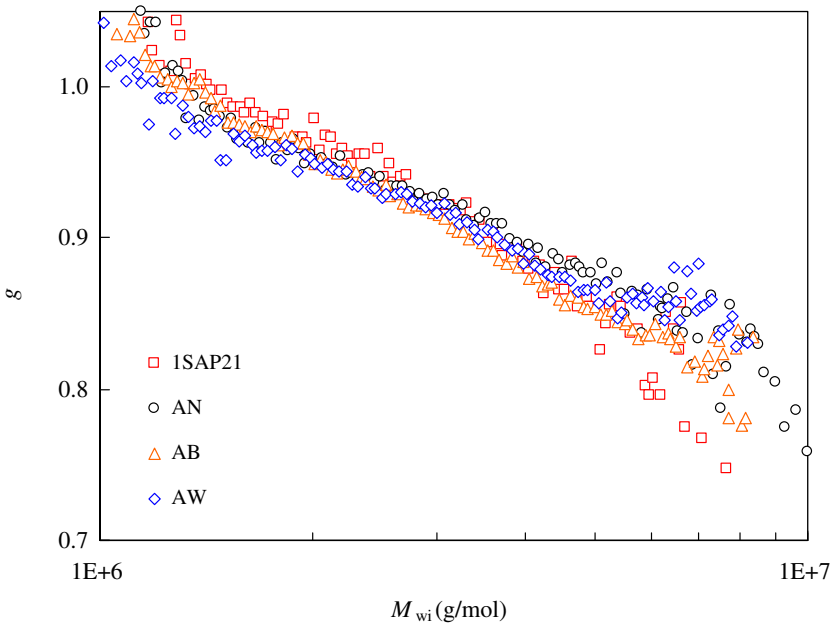

Fig. 5. Distributions of the contraction factor, *g*, for NR samples as a function of molar mass using linear standard PI as a reference.

compared to that of the standard linear PI means that their chains were assumed to be branched. Sample IR305, with the highest *v* value, had the least branched chains compared to the other SR samples. The Flory exponent gives a qualitative description of branching. For the comparison of the degree of branching between samples, taking a quantitative point of view, the contraction factor (*g*) and the number of branching points per chain (*m*) were used. Fig. 2 shows the distributions of *g* for SR samples as a function of molar mass using the standard linear PI as a reference. The calculations were performed using Astra with the equation of the conformation plot of standard linear PI ($R_g = 0.0135M_w^{0.606}$, Berry fit). The *g* of sample IR305 decreased to about 0.89 at M_{wi} 6000 kg/mol. The *g* of samples Natsyn2200 and Nipol2200 decreased to about 0.77 and 0.75 at M_{wi} 6000 kg/mol, respectively (Fig. 2) (Table 4). Of course, using sample IR305 as the linear reference, instead of the standard linear PI model, the *g* values of

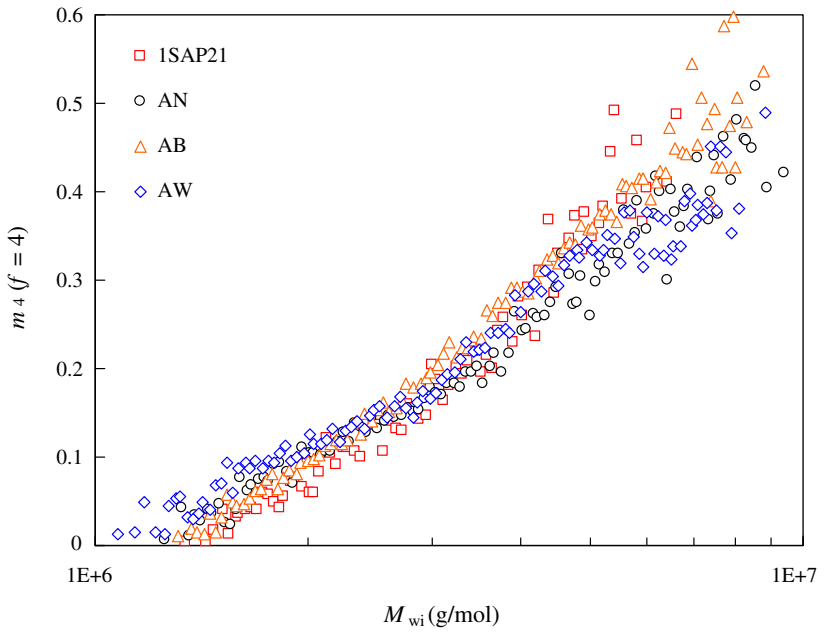

Fig. 6. Distributions of the number of branching per chain (m_4) for NR samples as a function of molar mass using linear standard PI as a reference.

the other SR samples were underestimated, increasing to about 10% (results not shown). Fig. 3 shows the number of branching points per chain for samples IR305, Natsyn2200 and Nipol2200 using the standard linear PI model as the linear reference and by assuming that the branching points were tetra-functional ($f = 4$). The number of branching points per chain (m_4) in the SR samples increased in line with the molar mass. Samples IR305, Natsyn2200 and Nipol2200 had respective m_4 values of about 0.26 ± 0.02, 0.70 ± 0.05 and 0.78 ± 0.05 for M_{wi} 6000 kg/mol (Table 4). Assuming that the branching points were tri-functional ($f = 3$), the distributions displayed the same tendencies as those of tetra-functional branching points (results not shown), but the number of branching points per chain (m_3) was increased by about 2.5 times compared to m_4 for the same molar mass.

Fig. 4 shows the conformation plots of NR samples, the straight line is the standard linear PI master curve used as the linear reference. The conformation plots of the samples displayed curvatures at low molar mass.

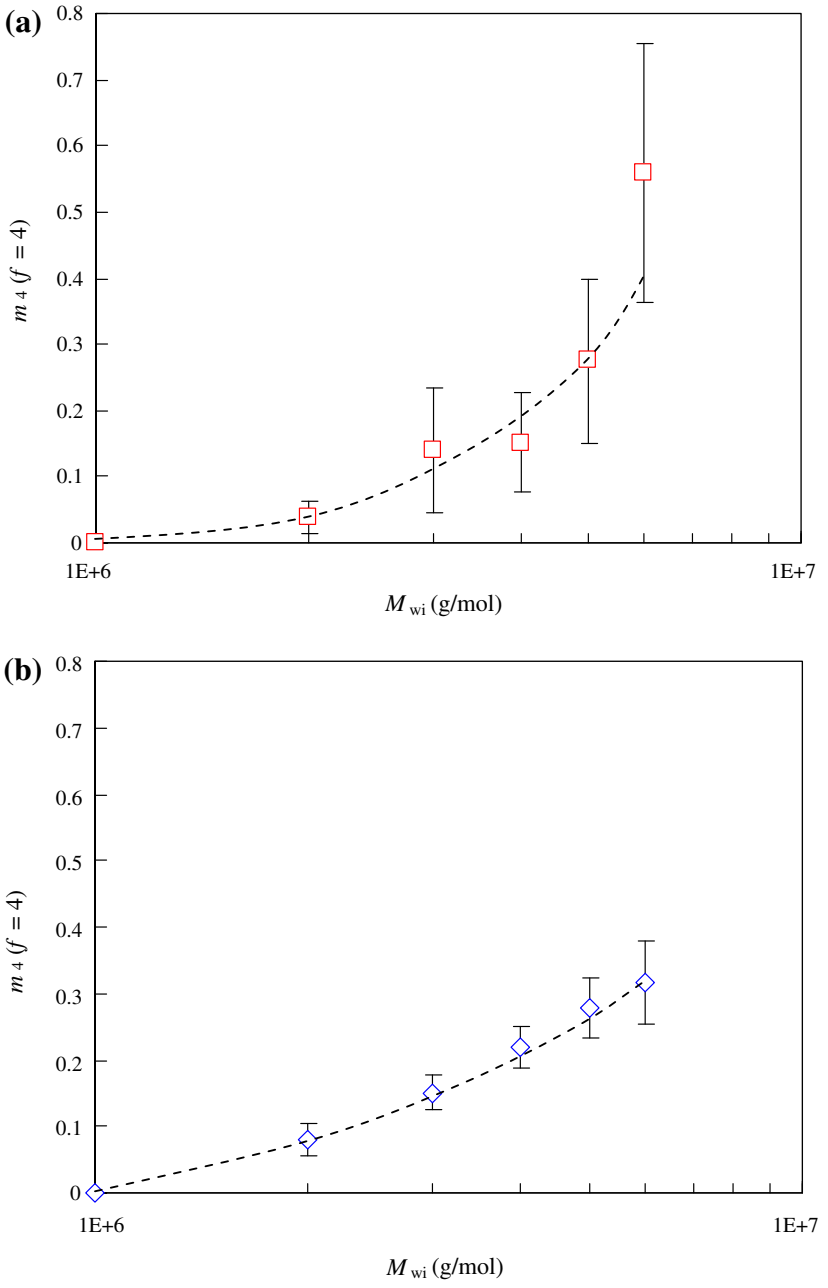

Fig. 7. Distributions of the number of branching per chain (m_4) for samples (a) 1SAP21 and (b) AW as a function of molar mass using linear standard PI as a reference (with 95% confidence intervals).

The curvatures depended on the type of NR and were caused by the abnormal elution phenomenon [12]. Samples 1SAP21 and AN displayed greater curvatures compared to samples AB and AW. For M_{wi} higher than 1000 kg/mol the conformation plots of the samples displayed conformation plots with gentler slopes compared to that of the linear reference. Fig. 5 shows the distribution of g for NR samples 1SAP21, AW, AB and AN as a function of molar masses. There was no significant difference in the distribution of g between the samples. Sample 1SAP21 will be considered later because of its particularity. For M_{wi} below 1000 kg/mol the g values were higher than unity (results not shown). This was caused by the curvature of the conformation plot. For M_{wi} higher than 1000 kg/mol, sample AW had a g of about 0.87 ± 0.02 at 6000 kg/mol, samples AB and AN had g of about 0.84 ± 0.05 and 0.88 ± 0.02, respectively (Table 4). These g values were obtained taking the closest injection of the Flory exponent (v) value given in Table 4, but it should be noted that a small error on v value, for example 1% for sample AW, g could vary from 0.85 to 0.89 (for M_{wi} = 6000 kg/mol). This variation led to a large variation in the number of branchings per chain (m) which it is nearly 20%. Indeed, m_4 (f = 4) of sample AW could vary from 0.26 to 0.38 for M_{wi} = 6000 kg/mol (Table 4). Fig. 6 shows the distributions of m_4 for NR samples as a

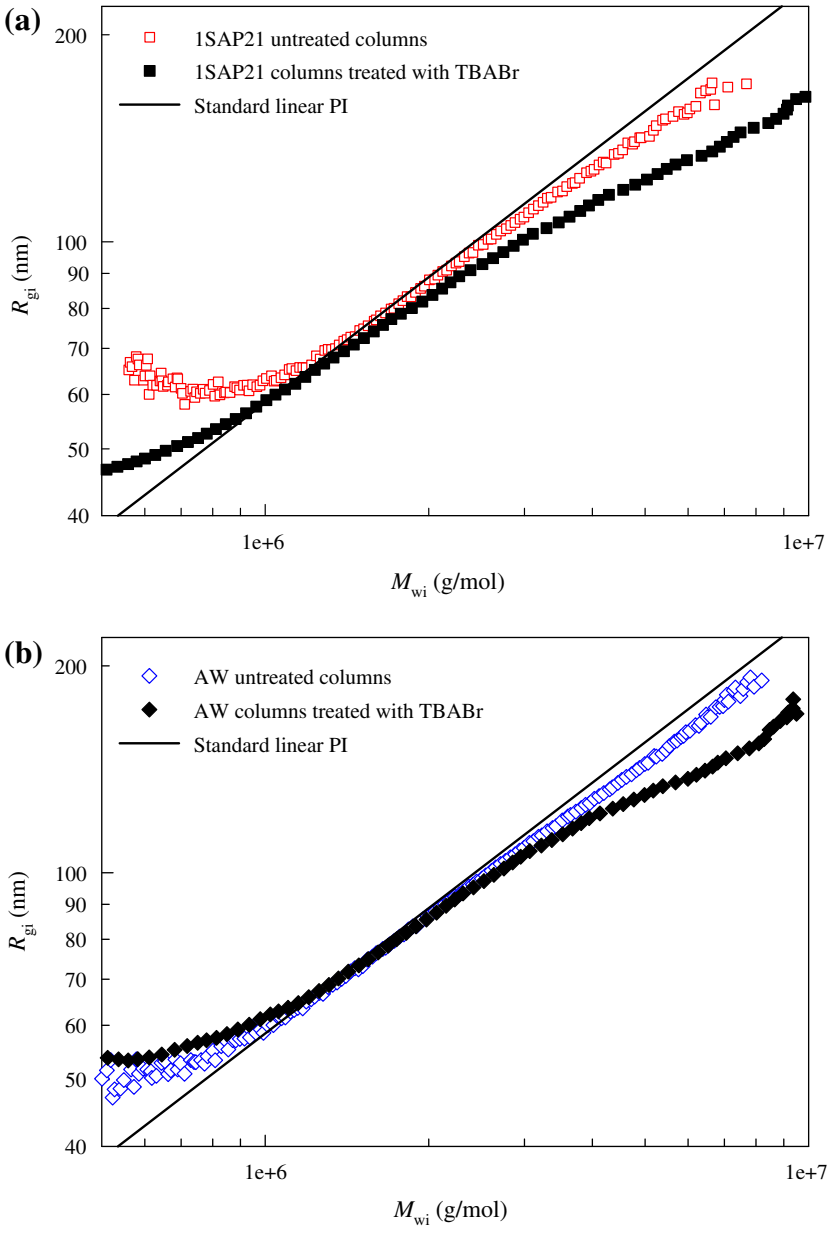

Fig. 8. Conformation plots of samples (a) 1SAP21 and (b) AW obtained with SEC-MALS using columns untreated and treated with TBABr before injection (the straight line is the conformation plot of the standard linear PI).

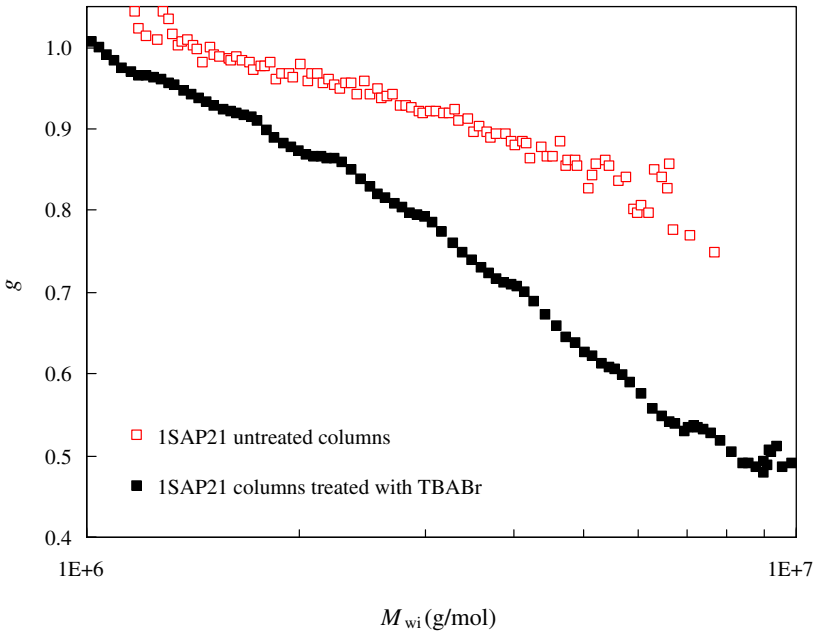

Fig. 9. Distributions of the contraction factor, *g*, for sample 1SAP21 obtained with SEC-MALS using columns untreated and treated with TBABr as a function of molar mass using linear standard PI as a reference.

function of molar mass. There was no significant difference in m_4 between the NR samples analysed. Samples AB and AW had a maximum value for m_4 of about 0.5 (at M_{wi} 6000 kg/mol) and 0.32 (at M_{wi} 6000 kg/mol), respectively (Table 4). For sample 1SAP21, it was better to give a range than an average value. Indeed, it can be seen from Table 4 that the *v* values for this sample were very variable and, consequently, out of 12 analyses (12 solutions prepared according experimental section over one year) we obtained *g* values ranging from 0.75 to 0.87 and m_4 values ranging from 0.37 to 0.75 at M_{wi} 6000 kg/mol, which was more variable compared to other samples (Table 4) (Fig. 7a and b). The lack of consistency for this sample was probably due to lower concentrations of the solutions injected because of a higher gel rate (Table 4). Nevertheless, although on raw rubber, all the NR samples analysed displayed very low m_4 (<1) compared to the literature ($0.6–2.4 < m_4 < 4.9–6.4$ [6,7]) but close to those for purified rubber [8]. This means that the chains appeared rather linear. The main reason for that should be the co-precipitation of nanoaggregates with higher chains during fractionation. Indeed, it can be seen on Fig. 1 of Ref. [19] that the higher fractions, unimodal before treatment with sodium methoxide, became bimodal after treatment with sodium methoxide. It is noteworthy that SEC-MALS analysis showed not significantly different *g* and m_4 for the NR samples (Figs. 5 and 6), but their gel rates were very different (Table 4), from 17% (AB) to 49% (1SAP21). The gel phase was removed from the solution by filtration prior to injection into SEC-MALS and could not therefore be analysed.

Due to nanoaggregates co-eluting with short-medium chains (<1000 kg/mol) [12], it was not possible to evaluate branching for this population. The only way to do that using SEC-MALS is to avoid the adsorption of nanoaggregates on the column packing as much as possible by adding tetrabutylammonium bromide (TBABr) to THF to treat the SEC columns and neutralise adsorption sites on the columns packing [12] (Fig. 8). For that, columns were flushed overnight with THF containing TBABr (3 g/L) then used with pure THF as mobile phase (samples were solubilised in pure THF before injection). The conformation plots for samples 1SAP21 and AW obtained with SEC-MALS using columns treated with TBABr displayed two slopes with an inflexion point at M_{wi} about 2500 kg/mol (Fig. 8). The conformation plots displayed lesser curvatures (less abnormal elution) than the one obtained using untreated columns, but abnormal elution was not completely eliminated. The slopes of the conformation plots for molar mass higher than 2500 kg/mol had Flory exponents equal to 0.38 after TBA treatment of the columns. With SEC-MALS using untreated columns, the nanoaggregates mainly co-eluted with medium chains due to adsorption on the column packing. Conversely, with SEC-MALS using columns treated with TBABr, the nanoaggregates mainly co-eluted with long chains and caused a decrease in the mean R_g of the slices of the chromatogram. Thus, for untreated columns, the *g* of sample 1SAP21 was between 0.75 and 0.87, though for treated columns *g* decreased to nearly 0.58 for M_{wi} 6000 kg/mol (Fig. 9). Consequently, the m_4 value increased from 0.37–0.75 (untreated columns) to 2.7 (treated columns) (results not shown). This means that slices of the chromatogram were a mixture of rather linear chains, as shown previously, and nanoaggregates. However, despite a much lower *g*, this difference could not be attributed to more branching.

4. Conclusion

This paper shows that the best way to determine the absolute number of branched points per chain (m) is to use Berry formalism and a model plot obtained with linear standard poly(*cis*-1,4-isoprene). The branching characteristics of natural rubber (NR) can only be determined by SEC-MALS for the higher molar masses ($1000 < M_w < 10{,}000$ kg/mol) because of nanoaggregates adsorbing onto the column packing and co-eluting with short-medium chains ($M_w < 1000$ kg/mol). Contrary to previous studies in the literature, our results suggest that the soluble long chains of the NR samples analysed had very low branching. This study showed that the soluble fraction of NR samples was composed of almost linear poly(*cis*-1,4-isoprene) macromolecules and nanoaggregates with assumed compact structure.

Acknowledgements

We should like to thank the French Embassy in Cambodia for funding the PhD grant, CIRAD DRS for financial support and Chup Rubber Plantation for assistance in obtaining natural rubber samples.

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