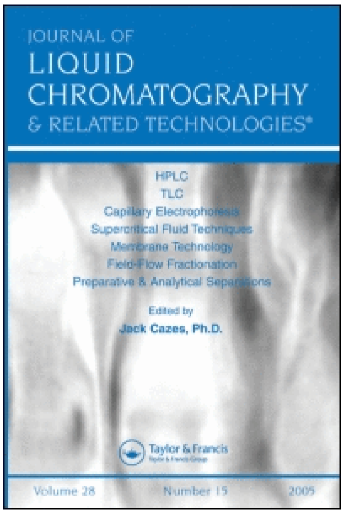

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF THE REFRACTIVE INDEX INCREMENT OF NATURAL AND SYNTHETIC POLY(*CIS*-1,4-ISOPRENE) SOLUTIONS AND ITS EFFECT ON STRUCTURAL PARAMETERS

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To cite this Article Kim, Chandy , Deratani, André and Bonfils, Frédéric(2010) 'DETERMINATION OF THE REFRACTIVE INDEX INCREMENT OF NATURAL AND SYNTHETIC POLY(*CIS*-1,4-ISOPRENE) SOLUTIONS AND ITS EFFECT ON STRUCTURAL PARAMETERS', Journal of Liquid Chromatography & Related Technologies, 33: 1, 37 — 45
To link to this Article: DOI: 10.1080/10826070903427072
URL: http://dx.doi.org/10.1080/10826070903427072

Journal of Liquid Chromatography & Related Technologies, 33:37–45, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903427072

DETERMINATION OF THE REFRACTIVE INDEX INCREMENT OF NATURAL AND SYNTHETIC POLY(*CIS*-1,4-ISOPRENE) SOLUTIONS AND ITS EFFECT ON STRUCTURAL PARAMETERS

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☐ *The refractive index increment* (d*n*/d*c*) *of a solution is a constant that indicates the variation of the refractive index with the solute concentration. It is used in the multi-angle light scattering technique to determine the concentration and the weight-average molar mass of polymers. It depends on many parameters, including the structure of the polymer, the solvent, the wavelength of the light and the temperature of the solution. To obtain accurate results, it is necessary to determine the* d*n*/d*c* *at the same condition as for the multi-angle light scattering measurement. In this work, the* d*n*/d*c* *at 633 nm of standard synthetic monodispere poly(*cis*-1,4-isoprene) (PI) and natural rubber (NR) solutions in THF were determined at 25 and 40°C using an Optilab DSP refractometer. The mean value of the* d*n*/d*c* *obtained for NR and PI was 0.13 mL/g. No significant difference between different types of samples and temperatures (25°C and 40°C) were observed. The nanoaggregates in NR solution that were not retained after filtration through 1 μm filter had no effect on the* d*n*/d*c*. *The intercept value of the conformation plot increased in line with the* d*n*/d*c*, *but the Flory exponent remained unchanged.*

Keywords natural rubber, polyisoprene, refractive index increment, size-exclusion chromatography with multi-angle light scattering

INTRODUCTION

For the multi-angle light scattering coupled either with size-exclusion chromatography or asymmetrical flow field-flow fractionation (SEC-MALS or A4F-MALS) the refractive index increment (dn/dc) of the solution is required. This constant indicates the variation of the refractive index with

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the concentration of the solution. It is used to convert the refractive index (RI) signal into the concentration. Then, both the concentration and the dn/dc values are needed by the light scattering detector (LS) to determine the weight-average molar mass (M_w) of the polymer. Since the dn/dc appeared as a squared term in the light scattering equation, the accurate value is therefore essential for the determination of the M_w.

The dn/dc depends on the chemical structure of the polymer,[1,2] the average molar mass,[3,4] the solvent, the wavelength of the light and the temperature.[5] The effect of temperature depended on the polymer-solvent pairs. The increase of dn/dc with temperature was rather low, in some cases even null. For a 10°C increase in temperature, the dn/dc increased by about 2 to 6%. The dn/dc was not affected by the chain branching of the polymer,[5] but it increases in line with the number average molar mass (M_n) until a limiting value.[6] Hadjichristidis and Fetters[4] showed that the dn/dc of poly(*cis*-1,4-isoprene) solution, in cyclohexane at 633 nm, reached its limiting value at $M_n \geq 10\,\mathrm{kg\,mol^{-1}}$. The d$n$/d$c$ increases in line with the increasing of the refractive index of the solvent (Eq. (1)) or the wavelength of the light (Gauchy equation Eq. (2)).

$$\mathrm{d}n/\mathrm{d}c = -\mathrm{A}\ n + \mathrm{B} \tag{1}$$

n is the refractive index of the solvent, A and B are constants.

$$\mathrm{d}n/\mathrm{d}c = \mathrm{A} + \mathrm{B}/\lambda^2 \tag{2}$$

λ is the wavelength of the light, A and B are Cauchy constants.

For the characterization of natural rubber (NR) and synthetic poly(*cis*-1,4-isoprene) (SR) using SEC-MALS, with detector MALS Dawn-DSP (Wyatt Technology),[7] the dn/dc at 633 nm in tetrahydrofuran (THF) is needed. THF was the most used solvent to solubilize NR samples because its polarity enables the reduction of the gel phase more than less polar solvents.[8,9] In the literature, the dn/dc values of NR and SR solutions were determined at wavelengths of 436 nm and 546 nm[10–12] (Table 1). The values vary from one author to another because the samples and the conditions used were different, especially the solvent. Extrapolating dn/dc values of Angulo-Sanchez et al.,[12] at 436 nm and 546 nm, using Eq. (2) gave $0.154\,\mathrm{mL\,g^{-1}}$ and $0.146\,\mathrm{mL\,g^{-1}}$ at 633 nm for NR and SR solutions in THF, respectively. A lower value of $0.124\,\mathrm{mL\,g^{-1}}$, at 633 nm, for guayule rubber was published in Polymer Handbook,[13] but the latter was referred to a wrong source. We supposed that it was the one of Hadjichristidis and Fetters[4] for synthetic monodisperse poly(*cis*-1,4-isoprene) in cyclohexane. Using Eq. (1), Reed and Urwin[11] showed that the dn/dc at 546 nm of a SR solution in a solvent could be predicted using the equation dn/d$c = -1.105\ n + 1.687$. From this equation, the dn/dc at 546 nm of a SR solution in THF

TABLE 1 Literature Values of dn/dc obtained for NR and SR Solutions in THF

Reference	Sample	Cell Temperature (°C)	dn/dc (mL g^{-1}) 436 nm	546 nm	633 nm
Schulz et al.[16]	NR[b]	25	0.149[e]	0.141[e]	0.137[f]
Vavra[10]	SR[c]	25	—	0.128	—
Reed and Urwin[11]	SR[c]	20	—	0.128[e]	—
Angulo-Sanchez et al.[12]	NR[b]	25	0.160	0.156	0.154[f]
	SR[c]	25	0.153	0.148	0.146[f]
Michielsen [13]?[a]	GR?[d]	25	—	—	0.124?

[a] Value published in Polymer Handbook was referred to a wrong source.

[b] Ribbed smoked sheet natural rubber.

[c] Industrial synthetic poly(*cis*-1,4-isoprene).

[d] Guayule rubber.

[e] Values obtained by extrapolation from other types of solvents using Eq. (1).

[f] Values obtained by extrapolation from other wavelengths using Eq. (2).

was 0.128 mL g^{-1}, which is in perfect agreement with Vavra.[10] The results published by Reed and Urwin[11] did not enable to determine the value of the dn/dc at 633 nm for a SR solution in THF, but according to Eq. (2), this value should be lower than the one of 546 nm (0.128 mL g^{-1}).

In this work, the dn/dc at 633 nm of synthetic poly(*cis*-1,4-isoprene) and NR solutions in THF at 25 and 40°C were measured using an Optilab DSP refractometer. The obtained value was validated against several standard monodisperse PI samples. The influence of the dn/dc on the conformation plots was also investigated.

EXPERIMENTAL

Samples

Standard monodisperse synthetic poly(*cis*-1,4-isoprene) (PI) and a natural rubber (NR) samples were used. The NR sample (AB) was a TSR5CV grade obtained with the latex from the clone PB217. The latex was treated with neutral hydroxylamine sulfate (0.15% w/w dry basis) prior to coagulation at pH 5.2 with formic acid. This grade of NR is more stable in structure and property than other normal grade. Standard PI samples with $M_{\rm w}$ ranging from 3.2 to 904 kg mol^{-1} were obtained from PSS (Polymer Standards Service) and were used as received.

Preparation of NR and PI Solutions

About 120 mg of the NR or standard PI samples were dissolved in 100 mL THF stabilized with 2,6-di-tert-butyl-4-methylphenol (BHT)

(100 mg/L). The NR solution was stored for 14 days in the dark, in a hot-water bath at 30°C, and stirred 1 hour daily during the last 7 days. For the standard PI samples, the solutions were stored only for 7 days and stirred 1 hour daily. The solution of NR was filtered through several 1 μm disposable filters (Acrodisc 1 μm, glass fiber, Pall) to remove gel phase. The filter was changed every 10 mL. From this solution, a series of solutions with 5 different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg mL^{-1}) were prepared by successive dilutions. As the gel phase was removed by filtration, the initial concentration of the solution was changed. Therefore, the filtered solution was subjected to a determination of the true concentration. For that, ten milliliters of solution was placed in an aluminum dish and was left to dry out the solvent in a fume cupboard overnight, then was vacuum dried at 40°C during 4 hours to obtain dried rubber. The aluminum dish with dried rubber was removed from the oven and placed in a desiccator for 1 hour before weighing.

d*n*/d*c* Measurement

The calibration of the cell of the Optilab DSP refractometer (Wyatt Technology, Santa Barbara, CA) at 633 nm was done at 25°C with NaCl solutions. As shown by Ehl et al.,[5] the variation of the calibration constant with temperature was lower than 1%, therefore we used the same constant for 25°C and 40°C. For the dn/dc measurements, the mobile phase was the same solvent used for the preparation of the solutions. A series of 5 different concentrations were injected for each sample solution using a manual injector with a 2 mL-loop and at a flow rate of 0.8 mL min^{-1} (Waters 515 HPLC pump). Each concentration was injected twice.

SEC-MALS Characterization and Theoretical Background

The samples (25 ± 5 mg) were dissolved in tetrahydrofuran (THF, 40 mL, HPLC grade) stabilized with 2,6-di-tert-butyl-4-methylphenol (BHT). The NR solutions were stored during 14 days in the dark, in a hot-water bath at 30°C, and stirred 1 hour daily during the last 7 days. For standard PI solutions, they were stored in a hot-water bath at 30°C for 7 days only, and stirred 1 hour daily. All the solutions were filtered through 1 μm disposable filters (glass fibre, Pall) prior to injection three times into SEC-MALS. The SEC equipment consisted of an online degasser (Elite^{TM}, 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 bed columns Mixed-A (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 $0.65\,\mathrm{mL\,min^{-1}}$; the injected volume was $150\,\mu\mathrm{L}$. The signals obtained with SEC-MALS detectors were analyzed with ASTRA software (version 5.3.1.5) (Wyatt Technology, Santa Barbara, CA) using Zimm fit method Eq. (3), by plotting $Kc/\Delta R(\theta)$ versus $\sin^2(\theta/2)$.

$$\frac{Kc}{\Delta R(\theta)_\mathrm{i}} = \frac{1}{M_\mathrm{wi}} + \frac{16\pi^2}{3\lambda_0^2}\frac{\left\langle R_\mathrm{g}^2\right\rangle_\mathrm{i}}{M_\mathrm{wi}}\sin^2(\theta/2) \tag{3}$$

$$K = \frac{4\pi^2 n_0^2}{N_\mathrm{A}\lambda_0^4}(\mathrm{d}n/\mathrm{d}c)^2 \tag{4}$$

$\Delta R(\theta)$ is the excess Rayleigh ratio, the ratio of scattered and incident light intensity; c is the solute concentration in $\mathrm{g\,mL^{-1}}$; K is an optical constant; θ is the light scattering angle; n_0 is the refractive index of the solvent; N_A is Avogadro's number and λ_0 is the wavelength of the laser beam in a vacuum.

RESULTS AND DISCUSSION

Determination of d*n*/d*c*

Table 2 shows values of dn/dc at 633 nm of NR and standard monodisperse synthetic poly(*cis*-1,4-isoprene) (PI) solutions in THF obtained from this work. In general, the dn/dc increased in line with the temperature,[5] but our results showed no significant difference in dn/dc between the cell temperatures of 25 and 40°C. The NR samples contain nanoaggregates even after filtration through 1 μm.[7] According to Tanaka et al.,[14] these

TABLE 2 Values of dn/dc at 633 nm of Standard Poly(*cis*-1,4-isoprene) and NR Solutions in THF Determined Using an Optilab DSP Refractometer[a]

Sample	Type	Description	dn/dc ($\mathrm{mL\,g^{-1}}$)
Cell temperature 25°C			
PI7	Synthetic PI	Standard monodisperse PI $M_\mathrm{w} = 270\,\mathrm{kg\,mol^{-1}}$	0.132
PI8	Synthetic PI	Standard monodisperse PI $M_\mathrm{w} = 590\,\mathrm{kg\,mol^{-1}}$	0.129
AB	NR	TSR5CV clone PB217	0.134
AB[b]	NR	TSR5CV clone PB217 lipids extracted	0.136
Cell temperature 40°C			
PI8	Synthetic PI	Standard monodisperse PI $M_\mathrm{w} = 590\,\mathrm{kg\,mol^{-1}}$	0.121
AB	NR	TSR5CV clone PB217	0.131
AB[b]	NR	TSR5CV clone PB217 lipids extracted	0.127

[a] Results obtained from this work.

[b] The sample lipids were extracted using chloroform/methanol mixture (1/2).

nanoaggregates are supposed to be composed of polyisoprene chains linked with proteins and phospholipids. The results showed that there was no significant difference in d*n*/d*c* between NR samples (raw and purified samples) and standard PI, which means that there was no effect of the nanoaggregates. This could be due either to the low concentration of the proteins residues or that the d*n*/d*c* of proteins in THF is close to $0.13\,\mathrm{mL\,g^{-1}}$. Arakawa and Kita[15] showed that d*n*/d*c* of proteins in water ($0.19\,\mathrm{mL\,g^{-1}}$) decreased by increasing the percentage of organic solvent to $0.11–0.14\,\mathrm{mL\,g^{-1}}$ in aqueous solution containing 80% of organic solvent.

All samples had d*n*/d*c* values varying from 0.121 to $0.136\,\mathrm{mL\,g^{-1}}$, with a mean value of $0.13\,\mathrm{mL\,g^{-1}}$, which was closed to the one published by Vavra[10] and Reed and Urwin,[11] at 546 nm, but very different from the one of Angulo-Sanchez[12] extrapolated at 633 nm.

Validation of the d*n*/d*c* Value

Several standard PI samples with M_w varying from 3.84 to $960\,\mathrm{kg\,mol^{-1}}$ were used to validate the value of d*n*/d*c* obtained from our experiments. The concentration and the M_w were used to check the validity of d*n*/d*c* value found ($0.13\,\mathrm{mL\,g^{-1}}$). The concentration is the most mastered parameter as we could determine it. For the M_w we have to trust the value given by the supplier. Therefore, in our opinion, the concentration is the most important parameter for checking the validity of d*n*/d*c* value. Figure 1 shows

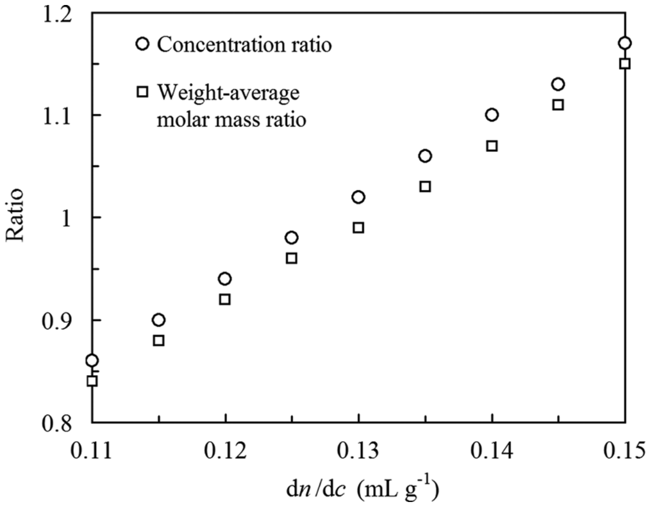

FIGURE 1 Plots of the concentration and weight-average molar mass (M_w) ratios (theoretical value divided by calculated value using SEC-MALS) as a function of d*n*/d*c* for the standard PI7 sample ($M_w = 270\,\mathrm{kg\,mol^{-1}}$).

TABLE 3 Comparison of Theoretical Values of M_w and Concentrations of Standard PI Samples with Values Obtained with SEC-MALS with $dn/dc = 0.13\,mL\,g^{-1}$

Standard sample	M_w ($kg\,mol^{-1}$)[a]				Concentration ($mg\,mL^{-1}$)[b]			
	Theory	Calculated	Difference	[c]	Theory	Calculated	Difference	[c]
PI2	3.8	3.2	15.8%	s	2.03	2.03	0%	ns
PI3	7.9	6.3	20.3%	s	2.04	2.05	−0.5%	ns
PI4	22.5	18.9	16.0%	s	1.54	1.57	−1.9%	ns
PI5	60.0	55.2	8.0%	ns	1.54	1.56	−1.3%	ns
PI6	108	109	−0.9%	ns	1.35	1.37	−1.5%	ns
PI7	270	272	−0.7%	ns	1.00	1.02	−2.0%	ns
PI8	590	620	−5.1%	ns	0.82	0.84	−2.4%	ns
PI9	963	904	6.1%	ns	0.78	0.81	−3.8%	ns

[a] Weight-average molar mass, the theoretical values were provided by supplier.

[b] The theoretical concentration were those prepared for SEC-MALS injections.

[c] s or ns: the difference between theoretical and calculated values were significant ($P < 0.01$) or not significant.

the concentration and the M_w ratios (theoretical values divided by the calculated values obtained with SEC-MALS) of PI7 as a function of the dn/dc. The concentration and M_w ratios about one were obtained for the dn/dc of $0.13\,mL\,g^{-1}$. This means that the latter was a suitable value for PI7. For all standard PI samples, the calculated concentrations were not significantly different from the theoretical values (Table 3). Moreover, the calculated M_w of all standard PI samples were not significantly different from the theoretical ones, except PI2, PI3 and PI4. For PI2 and PI3, part of the difference between calculated and theoretical values of M_w can be explained by low M_w which were close to the limit value given by Hadjichristidis and Fetters ($10\,kg\,mol^{-1}$).[4] Indeed, taking into account their results, for PI2 (M_w $3.8\,kg\,mol^{-1}$), the dn/dc should be about 3% lower than $0.13\,mL\,g^{-1}$, so about $0.126\,mL\,g^{-1}$. With this dn/dc value, M_w was equal to $3.5\,kg\,mol^{-1}$ instead of $3.2\,kg\,mol^{-1}$.

Effect of the d*n*/d*c* on the Conformation Plot

The M_w and radius of gyration (R_g) were parameters that are used for determining the conformation of polymer chains in solution. The equation of the conformation plot is $R_g = A\ M_w^{\nu}$, where A is a constant and ν is the Flory exponent.[17] The dn/dc had an influence on M_w, but not on R_g. The latter is angular dependant and thus depends only on the slope of the fit curve $Kc/\Delta R(\theta)$ (Zimm fit). Indeed, the variation in dn/dc had no effect on the slope since the terms $Kc/\Delta R(\theta)$ for all angles varied proportionally in line with the dn/dc Eq. (3 and 4). The equations of the conformation plots obtained with dn/dc $0.11\,mL\,g^{-1}$, $0.13\,mL\,g^{-1}$ and $0.15\,mL\,g^{-1}$

were $R_g = 0.0156M_w^{0.592}$, $R_g = 0.0172M_w^{0.592}$ and $R_g = 0.0187M_w^{0.592}$, respectively. The dn/dc had an influence on A but not on ν.

CONCLUSION

In this study, we showed that there was no significant difference in dn/dc between different types of natural rubber and standard monodisperse poly(*cis*-1,4-isoprene) samples, neither between the temperatures of the tests (25 and 40°C). The mean value of dn/dc at 633 nm of standard monodisperse synthetic poly(*cis*-1,4-isoprene) and natural rubber solutions in THF was $0.13\,\mathrm{mL\,g^{-1}}$. This value was validated against several standard PI samples with M_w varying from 3.84 to $960\,\mathrm{kg\,mol^{-1}}$. The d$n$/d$c$ had an influence on the conformation plot, the intercept varied in line with the dn/dc, but the Flory exponent remained unchanged. The nanoaggregates had no significant influence on the dn/dc of natural rubber as it is rather closed to the one of standard synthetic poly(*cis*-1,4-isoprene) samples.

ACKNOWLEDGMENT

We thank Ms. Valérie Bonniol of the European Membrane Institute, University of Montpellier 2, for her assistances.

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