Steric Exclusion Chromatography Study of Natural Rubber Films Prepared from Fresh Field Latex

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Cyclohexane was used to replace tetrahydrofuran (THF) as the elution phase in analysis of natural rubber by steric exclusion chromatography The films, which were prepared from fresh field latex and dissolved in cyclohexane, had high gel rates up to 50% in some cases and overestimated average molecular weights These problems were linked to certain hydrophilic non-rubber constituents and more particularly to the crosslinking they seem to bring about under certain conditions This study showed that these crosslinking, which are not covalent, could be overcome by adding hydroxylamine neutral sulphate to the latex prior to film preparation, or by washing the films in deionised water before dissolving them. Using a mixed solvent, such as a cyclohexane and THF mixture (95/5, %v/v), to dissolve this type of samples also significantly limited the proportion of gel.

Natural rubber, a polymer of very high molecular weight, is mostly obtained from *Hevea brasiliensis*. As a polymer, its processing[1], mechanical or elastic[2] properties will largely depend on the mean chain length. The most conventional way of determining this value is to calculate average molecular weight of the polymer sample. Using steric exclusion chromatography (SEC), one can access: number-average molecular weight $(\overline{M}_n)$; weight-average molecular weight $(\overline{M}_p)$; z-average molecular weight $(\overline{M}_z)$ and of course the distribution of molecular weights.

$\overline{M}_n$ is defined as the weight of the sample in grams (ΣW_i, or $\Sigma N_i M_i$), divided by the total number of chains present (N, or ΣN_i). Here, W_i and N_i are the weight and number of molecules of molecular weight M_i, respectively, and i the

increment on the entire distribution of molecular weights. Thus, $\overline{M}_n$ is given by *Equation 1* and can be calculated from the SEC data by *Equation 2*, as shown below:

$$\overline{M}_n = \frac{\sum N_i M_i}{\sum N_i} \qquad \ldots 1$$

$$\overline{M}_n = \frac{\sum h_i}{\sum h_i / M_i} \qquad \ldots 2$$

h_i is the SEC curve height corresponding to the ith retention volume (or time) increment and M_i the molecular weight of the compound eluted at the ith retention volume. The equation assumes that h_i values are proportional to the

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solute concentration and M_i is sampled in equal volume increments. Likewise, $\overline{M}_p$ and $\overline{M}_z$ are defined by *Equations 3* and *4* and can be determined in SEC by *Equations 5* and *6*.

$$\overline{M}_p = \frac{\sum N_i M_i^2}{\sum N_i M_i} \qquad ... 3$$

$$\overline{M}_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2} \qquad ... 4$$

$$\overline{M}_p = \frac{\sum h_i M_i}{\sum h_i} \qquad ... 5$$

$$\overline{M}_z = \frac{\sum h_i M_i^2}{\sum h_i M_i} \qquad ... 6$$

The polydispersity index *(I)*, which indicates the extent of molecular weight distribution, is defined as ratio: $\overline{M}_p / \overline{M}_n$.

Subramaniam[3-4], at the beginning of the 1970s, was the first to study natural rubber by SEC. He was also the only person to study clone effects on molecular weight distribution and to discover bimodal distributions (type 1 and type 2) for most of the clones studied, and distributions with a shoulder in the low weights (type 3) for some clones *(Figure 1)*. Subsequently, many authors[5-14] also became interested in SEC analysis of natural rubber. In most cases, tetrahydrofuran was used as the elution solvent, except by Bartels *et al.*[10] who used cyclohexane.

Using tetrahydrofuran for SEC analysis of polyisoprenes can be a problem due to peroxides that form quite easily. Indeed, these peroxides can trigger degradation and/or crosslinking of polydienic chains. There is also another drawback with tetrahydrofuran — it is highly hygroscopic, which can cause problems during natural rubber analysis in a tropical environment. In the best of cases, this phenomenon will modify hydrodynamic volume of the macromolecules[14]; in the worst case, it can cause precipitation of the polyisoprenic chains.

On the other hand, cyclohexane is not very hygroscopic and peroxide formation is only very slight, hence it is not necessary to add an antioxidant to the elution phase, and UV detection can be used at 220 nm. It is essential to use a UV detector since it is more sensitive than a refractometer. This is important, as natural rubber is a polymer of very high molecular weight, so it is advisable to inject low-concentration solutions of around 0.2 mg/ml[8] in order to overcome viscosity effects. For this reason, the chromatograms obtained are tricky to use and can be a source of poor repeatability if a refractometer is used, however sensitive.

For these reasons we also preferred to use cyclohexane as the elution phase for a SEC study of natural rubber to avoid all these potential causes in the variability of result.

In order to monitor changes in average molecular weights during the different processing stages, it is necessary to know their values as they leave the tree. Like Subramaniam[4-5], we decided to prepare films for analysis from fresh field latex. We describe below the different points that led us to select the optimum film preparation procedure, and SEC analysis of this type of samples.

MATERIAL AND METHODS

Films Preparation

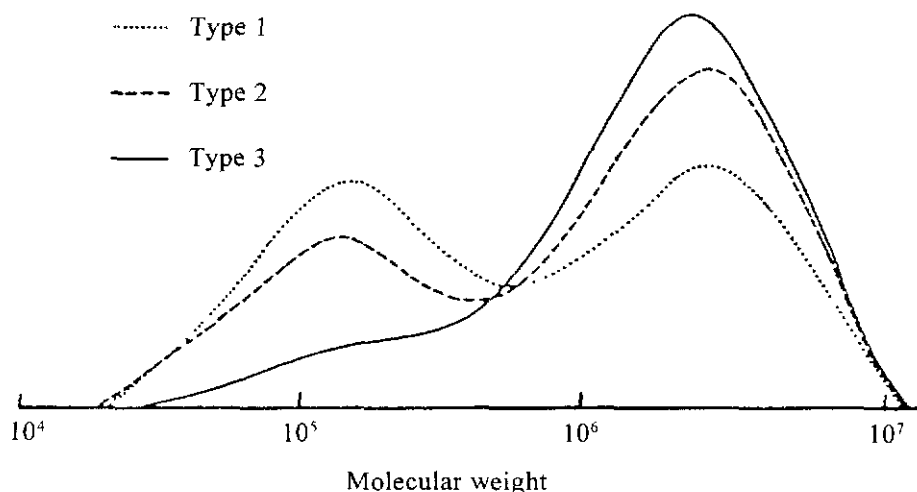*Unwashed films*. Around 40 drops of fresh field latex, with or without added hydroxy-

Figure 1. Types of molecular distribution curves obtained for natural rubber (Subramaniam[3])

lamine neutral sulphate (HNS) were deposited on a glass plate. The latex was spread out with a glass slide, then blown dry with compressed air, or with a cold hair drier for 30 min at room temperature, then in a vacuum oven at 40°C for 4 h. The films were then placed overnight in a desiccator containing silica gel.

Washed films. Around 40 drops of fresh field latex were deposited on a glass plate. The latex was spread out with a glass slide, then blown dry with compressed air, or with a cold hair drier, for 30 min at room temperature. The films were washed by immersion in deionised water at 50°C for 30 min, dried again with compressed air for 30 min, then in a vacuum oven at 40°C for 4 h. The films were then placed overnight in a desiccator containing silica gel. The drying conditions used give a volatile-matter content of around 0.3% with this type of samples. Films were stored in vials in nitrogen and kept in the dark prior to solution preparation.

Preparation of Solutions Analysed

The films were dissolved in cyclohexane stabilised with 2,6-di-tert-butyl-4-methylphenol (BHT), at a rate of 120 mg for 30 ml of solvent. The solutions were gently stirred periodically by hand for 5 days, then centrifuged (17 000 r.p.m., *i.e.* 35 000 g for 1 h at 17°C), diluted to 0.2 mg/ml and filtered (porosity 0.45 μm) and injected in SEC apparatus.

Determination of Gel Rate

After centrifugation of the natural rubber solution, 10 ml of supernatant were drawn off and placed in an aluminium evaporating dish and the cyclohexane was evaporated overnight in a fume-cupboard. The dish was then placed in a vacuum oven at 40°C for 4 h. Dry extract obtained in this way was used to calculate the gel percentage.

Apparatus

The chromatograph used consisted of an ERMA ERC-3112 solvent gas remover, a Waters 510 pump, an automatic injector, a Waters 486 UV detector (220 nm), a Waters RI410 refractometer, and two PLGEL 30 cm mixed columns with a porosity of 20 μm (Polymer Laboratories). The entire installation was computer controlled by special software (Maxima-Waters).

The column temperature was fixed at 55°C. The cyclohexane flow rate was 0.8 ml/min, the injected volume 200 μl (at a concentration of around 0.2 mg/ml), for the natural rubber samples, and 20 μl for the standard solutions. Calibration was carried out with synthetic poly(cis-isoprenes) with molecular weights of 1310, 3030, 8000, 32 400, 71 950, 97 200, 295 000, 452 000, 1.2 million and 4.4 million (expressed in g/mole). Prior to injection, the solutions were filtered (porosity of 0.45 μm).

The centrifuge used was a SORVAL RC5B; the solvents were of 'ANALAR' quality and were filtered through a filter (porosity of 0.45 μm) before use in SEC.

RESULTS AND DISCUSSION

Two clones were studied: RRIM 600 and AVROS 2037. The first films, prepared from fresh field latex, were not subjected to any subsequent processing (washing or HNS treatment). The results obtained (*Table 1* and *Figure 2*) clearly show bimodal distribution for RRIM 600 and unimodal distribution with a shoulder for AVROS 2037. Nevertheless, the gel rates, particularly for AVROS 2037, were particularly high.

As the films studied were stored for 133 days prior to analysis, it was reasonable to assume that storage hardening, a well-known phenomenon with natural rubber, was responsible for the very high gel rate. However, in the case of AVROS 2037, the gel rates were consistently around 50% in cyclohexane (*Table 2*), irrespective of the film storage time (22 to 182 days) prior to solution preparation. On the other hand, when the solution was prepared with a cyclohexane/THF mixture (95/5), the same films only contained 10% to 27% of gel. On the whole, these results (*Table 2*) show that variations in gel quantity should be interpreted with caution. Indeed, this phenomenon is partly linked to storage hardening — numerous hypotheses[12,16,17] have been put forward to explain it, and also to the type of solvent used to dissolve the sample[15].

Whatever the case, it is very important to prepare films from fresh field latex that were not subject to variation over time (storage hardening; oxidation), so that samples stored for 2 to 3 weeks, if necessary, could be analysed by SEC, and to obtain results as close as possible to those obtained with fresh films. To this end, we either added hydroxylamine neutral sulphate (HNS), which is well known for its action in preventing storage hardening, to the latex prior to film preparation, or washed the films in deionised water at 50°C. Each treatment was replicated 3 times for each clone and each replicate, dissolved in pure cyclohexane, was injected three times into the SEC apparatus. An analysis of variance on these results revealed different responses to the treatments. Compared with the control treatment (no HNS; no washing), HNS addition (3 g/kg of dry rubber) to the latex prior to film preparation led to a reduction in the gel fraction for both clones, a significant drop in the number-average molecular weight (M_n), the weight-average molecular weight (M_p) and the z-average molecular weight (M_z), an equally significant increase in the polydispersity index (I) as shown in *Tables 3* and *4*.

TABLE 1. RESULTS OBTAINED FOR RAW FILMS PREPARED FROM
FRESH FIELD AVROS 2037 AND RRIM 600 LATEX

Sample[a]		'Gel' %	M_z ($\times 10^{-3}$) (PI eq.)[b] (g $\times$ mol^{-1})	M_p ($\times 10^{-3}$) (PI eq.)[b] (g $\times$ mol^{-1})	M_n ($\times 10^{-3}$) (PI eq.)[b] (g $\times$ mol^{-1})	I
AVROS 2037 (Batch 3)	Film 1	47.0	3 079	1 498	198	7.56
	Film 2	53.4	3 142	1 527	198	7.71
	Film 3	35.0	3 047	1 473	201	7.33
	Mean	45.1	3 089	1 499	200	7.50
	S.d.[c]	9.3	48	27	2	0.19
RRIM 600 (Batch 3)	Film 1	26.5	3 136	1 284	146.6	8.78
	Film 2	30.0	3 223	1 353	153.7	8.84
	Film 3	28.5	3 182	1 317	148.5	8.80
	Mean	28.3	3 179	1 319	150	8.81
	S.d.	1.8	44	35	4	0.03

[a]The films were stored for 133 days before being dissolved in pure cyclohexane
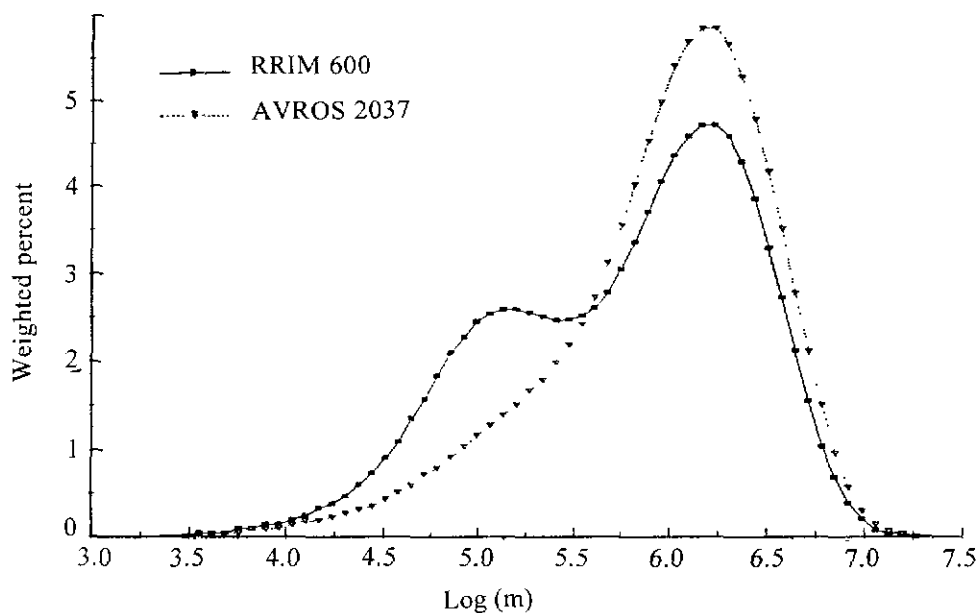[b]Polyisoprene equivalent
[c]Standard deviation

Figure 2. Molecular distribution curves obtained for raw films
prepared from fresh latices.

TABLE 2. VARIATION IN GEL QUANTITY DEPENDING ON SOLVENT POLARITY
AND FILM STORAGE TIME (FILMS NOT WASHED; WITHOUT HNS)

| Sample | Film storage time (days) | Gel % depending on dissolving solvent | | | |
		Cyclohexane	Cyclohexane + 5% THF	THF	
AVROS 2037		50.5	10.0	–	Mean
Batch 1	22–26	9.6	1.0	–	S.d.[b]
(2/04/93)[a]					
AVROS 2037		55.0	17.8	15.9	Mean
Batch 2	54–94	10.2	1.5	–	S.d.
(8/06/93)					
AVROS 2037		45.1	–	–	Mean
Batch 3	133	9.3	–	–	S.d.
(14/12/93)					
AVROS 2037		53.0	27.0	23.7	Mean
Batch 3	182	–[c]	2.2	1.0	S.d.
(14/12/93)					

[a]Latex collection date
[b]S.d.: Standard deviation
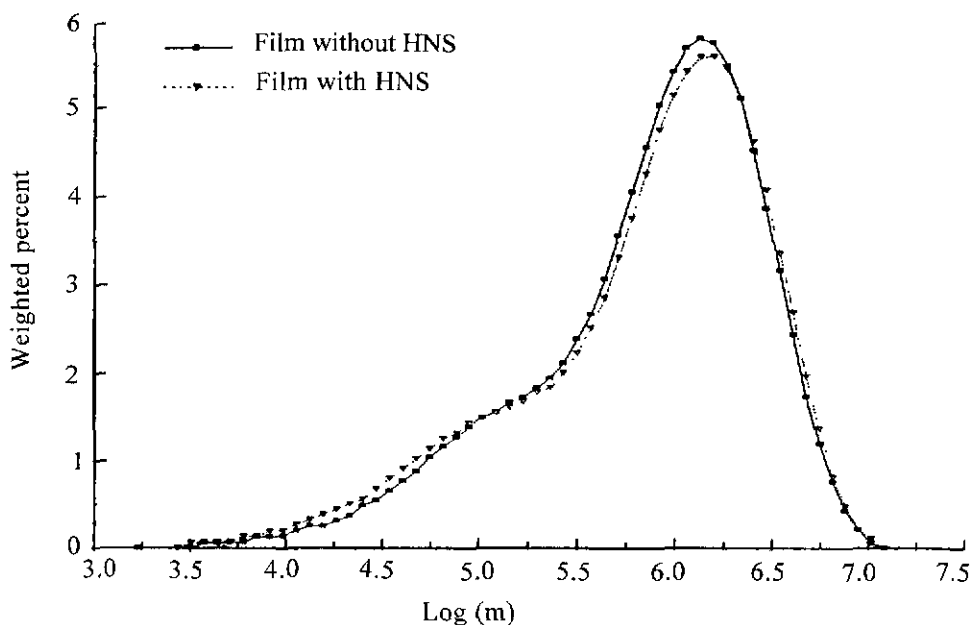[c]Standard deviation is not given, as only one film was tested

Figure 3. Molecular distribution curves for unwashed films
prepared from fresh AVROS 2037 latex.

TABLE 3. RESULTS OBTAINED FOR FILMS PREPARED FROM FRESH FIELD
AVROS 2037 LATEX (BATCH 3) AND DISSOLVED IN PURE CYCLOHEXANE

Sample[a]		'Gel' %	$M_z (\times 10^{-3})$ (PI eq.)[a] $(g \times mol^{-1})$	$M_p (\times 10^{-3})$ (PI eq.)[a] $(g \times mol^{-1})$	$M_n (\times 10^{-3})$ (PI eq.)[a] $(g \times mol^{-1})$	I
AVROS 2037 Not washed/ no HNS	Film 1	47.0	3 079	1 498	198	7.56
	Film 2	53.4	3 142	1 527	198	7.71
	Film 3	35.0	3 047	1 473	201	7.33
	Mean	45.1	3 089	1 499	200	7.50
	S.d.[b]	9.3	48	27	2	0.19
AVROS 2037 Not washed/ with HNS 3[c]	Film 1	22.9	2 984	1 416	162	8.74
	Film 2	23.6	3 029	1 456	175.4	8.30
	Film 3	23.0	2 932	1 374	153	8.98
	Mean	23.2	2 982	1 415	163	8.67
	S.d.	0.4	49	41	11	0.34
AVROS 2037 Washed/no HNS	Film 1	22.0	2 711	1 318	204	6.46
	Film 2	19.5	2 915	1 414	199	7.11
	Mean	20.8	2 813	1 369	202	6.78
	S.d.	1.8	145	68	4	0.46

[a]Polyisoprene equivalent
[b]Standard deviation
[c]G/kg of dry rubber

Washing the films also resulted in a highly significant drop in the gel rate, which was greater than for the treatments with HNS. For both clones, there was also a highly significant reduction in M_p and M_z compared to the control treatment (no HNS; no washing) as revealed in *Tables 3* and *4*. For AVROS 2037, M_n and I remained around the same as the values obtained with the control treatment; the same applied to the RRIM 600 poly-dispersity index (I).

The two treatments studied also had a substantial effect on molecular weight distribution, the effect being greater for RRIM 600 (*Figures 3* to *6*). The polydispersity indices obtained by adding HNS to the latex were much higher than those obtained with untreated films. This was due to an increase in low weight populations and it would therefore seem that polyisoprene chains participated in crosslinking, or were retained in the gel part, irrespective of their size.

This drop in the gel rate, M_p and M_z therefore means that the treatments – HNS addition or film washing – cancel out part of the crosslinking seen with raw films dissolved in pure cyclohexane. These crosslinks are therefore at the origin of an increase in gel rate and an overestimation of the M_p and M_z values. It also means that the crosslinks tend to persist even after the cyclohexane solution has been diluted (0.2 mg/ml). The energy of the bonds

TABLE 4. RESULTS OBTAINED FOR FILMS PREPARED FROM FRESH FIELD
RRIM 600 LATEX (BATCH 3) AND DISSOLVED IN PURE CYCLOHEXANE

Sample[a]		'Gel' %	M_z ($\times 10^{-3}$) (PI eq.)[a] (g $\times$ mol^{-1})	M_p ($\times 10^{-3}$) (PI eq.)[a] (g $\times$ mol^{-1})	M_n ($\times 10^{-3}$) (PI eq.)[a] (g $\times$ mol^{-1})	I
RRIM 600 Not washed/ no HNS	Film 1	26.5	3 136	1 284	146.6	8.78
	Film 2	30.0	3 223	1 353	153.7	8.84
	Film 3	28.5	3 182	1 317	148.5	8.80
	Mean	28.3	3 179	1 319	150.0	8.81
	S.d.[b]	1.8	44	35	4.0	0.03
RRIM 600 Not washed/ with HNS 3[c]	Film 1	23.4	3 107	1 272	101.4	12.90
	Film 2	20.0	3 083	1 260	109.2	11.50
	Film 3	24.0	3 043	1 224	90.5	13.50
	Mean	22.5	3 078	1 252	100.4	12.60
	S.d.	2.2	32	25	9.0	1.00
RRIM 600 Washed/no HNS	Film 1	10.2	3 009	1 168	122.2	9.60
	Film 2	11.5	3 007	1 171	123.6	9.50
	Film 3	11.2	3 011	1 198	124.8	9.60
	Mean	11.0	3 009	1 179	123.5	9.50
	S.d.	1.0	2	17	1.0	0.10

[a]Polyisoprene equivalent
[b]Standard deviation
[c]G/kg of dry rubber

behind this crosslinking must therefore be relatively strong for them not to be destroyed after substantial dilution, whereas simple Van der Waals interactions or entanglements would be overcome. Many hypotheses have been put forward to explain origins of crosslinks in natural rubber. Covalent crosslinks[16] and/or lower energy bonds, hydrogen bonds[12] or metallic-ligand cation type bonds[17], have been claimed to form between the polyisoprene chains and certain non-rubber constituents. The compounds most frequently blamed are amino acids and proteins. Whatever the case, the products favouring such crosslinks in films prepared from fresh field latex are highly hydrophilic and their action can be inhibited by HNS or film washing.

Adding 5% THF to the cyclohexane (*Table 2*) when dissolving films that are neither treated with HNS nor washed, also resulted in a significant drop in the gel rate. It is therefore unlikely that the crosslinks responsible for the high gel contents in pure cyclohexane, though apparently very strong, are covalent. One possible explanation for their persistence in pure cyclohexane may lie in the very weak polarity of such a solvent. Adding THF
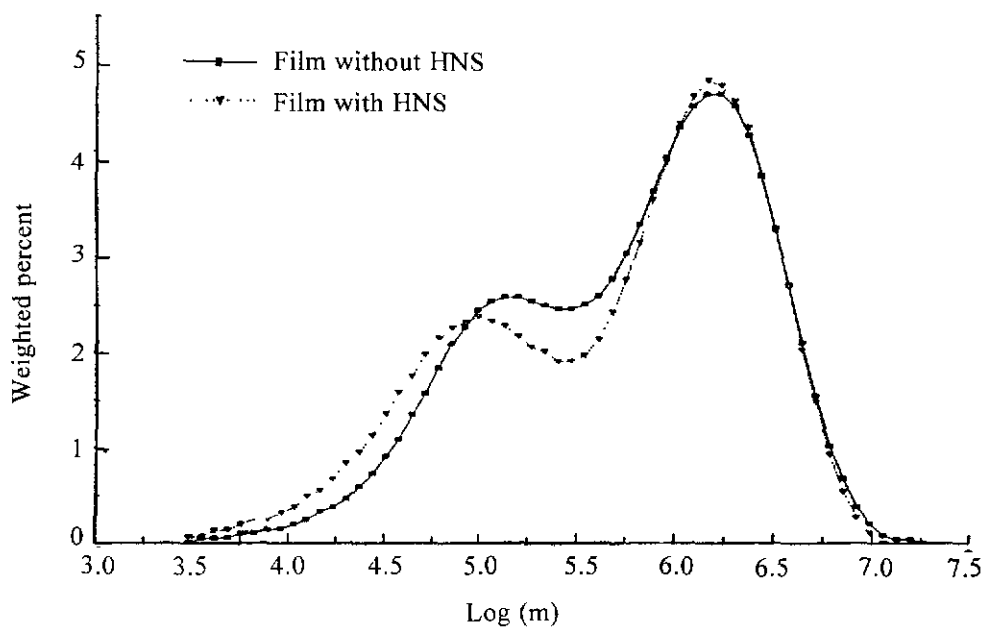
Figure 4. Molecular distribution curves obtained for unwashed films prepared from fresh RRIM 600 latex.
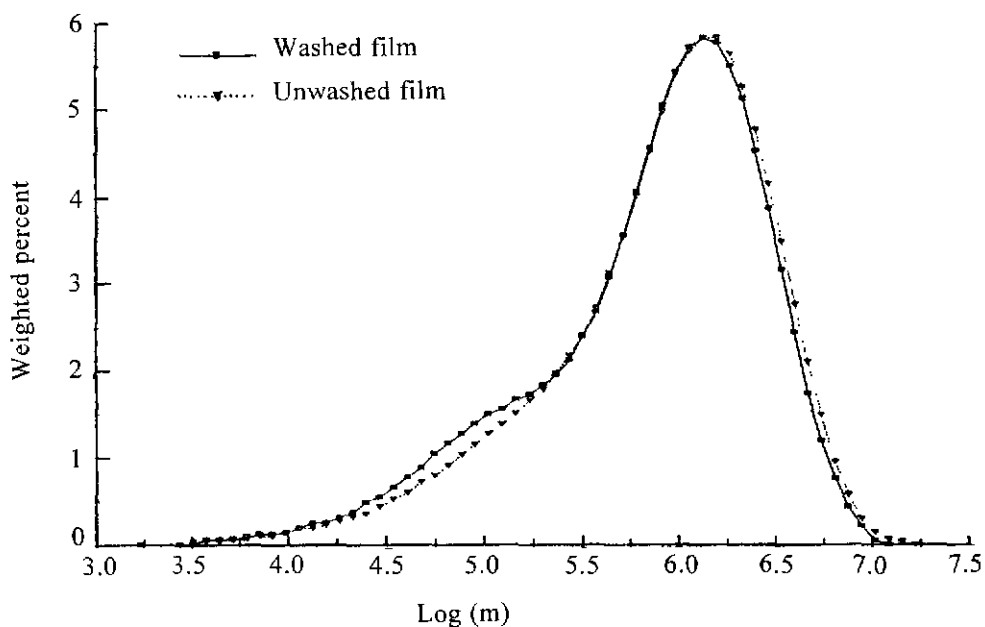

Figure 5. Molecular distribution curves obtained for films without HNS prepared from fresh AVROS 2037 latex.
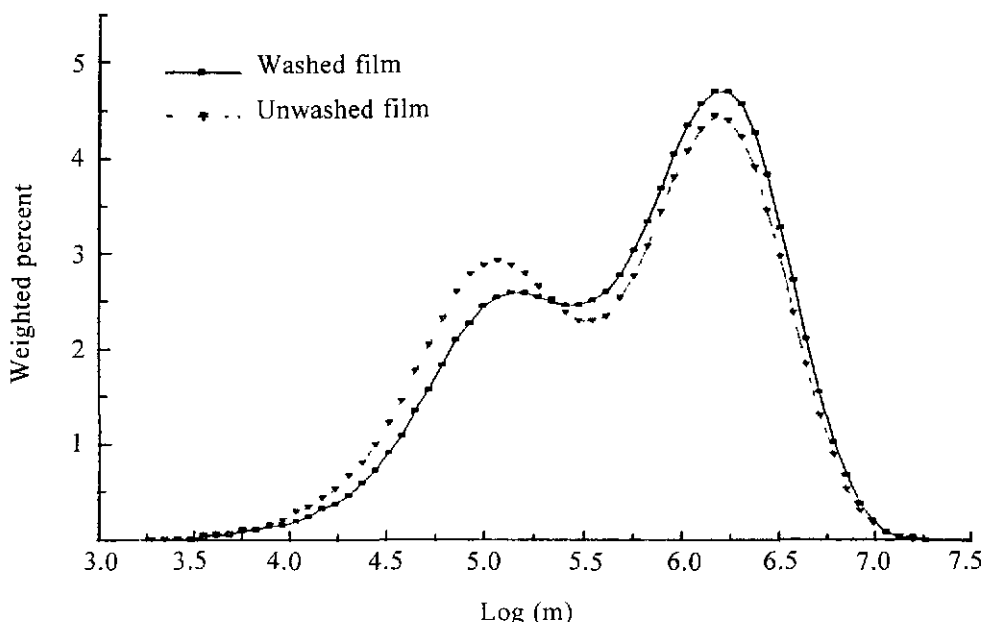
Figure 6 Molecular distribution curves obtained for films without HNS prepared from fresh RRIM 600 latex

increases solvent polarity and would therefore seem to 'break' certain interactions between highly polar compounds, thereby opening up the zones inaccessible to the cyclohexane.

CONCLUSION

We were able to show through this study that natural rubber samples taken from fresh field latex can be analysed by SEC using cyclohexane as the elution phase. However, in such SEC conditions, it is preferable to wash the films in water and/or use a cyclohexane/THF mixture (95/5,%v/v) to dissolve the samples to avoid overestimation of M_p and M_z values. Overestimation of molecular weights would seem to be linked to the existence of highly hydrophilic compounds, which are at the origin of non-covalent crosslinks, but whose energy is greater than simple physical interactions. These bonds are not 'broken' by cyclohexane, even after dilution. Such

interactions may be ionic and, depending on the polarity of the solvent, the well-known ionic polymerisation concepts of slack and linked ion pairs would seem to occur. It would also be reasonable to assume that they are hydrogen bonds. It is in fact highly probable that a combination of the above two interactions occurred within the samples analysed.

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