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Novel Insight into the Gel Phase of Hevea *Natural Rubber*

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In a suitable organic solvent, Hevea *natural rubber (NR) disintegrates into an insoluble gel phase (macrogel) and a sol phase consisting of polyisoprene chains with micro-aggregates (microgel). Studies on* Hevea *microgel following the advent of its quantitation by steric exclusion chromatography (SEC) are quite recent. In this study, transesterification of NR favoured macrogel elimination with unclear effects on the microgel. The NR's structure and the relative gel quantities varied in solution (depending on the solvents used) and during transesterification to produce substantial quantities of low-to-average molar mass chains (probably from the macrogel and/or the microgel fraction). The macrogel broke down equally to microgel. This microgel, representing 25%–30%* Hevea *rubber, remained insensitive to transesterification. The NR's structure was made up essentially of variably-sized membrane-delimited entities. On dissolution, these entities were denatured and through this liberated more or less branched polyisoprene chains, as well as some microgel and macrogel.*

Keywords: Natural rubber; microgel; steric exclusion chromatography

Natural rubber (NR) from *Hevea brasiliensis* differs from most synthetic homologues through its more complex structure, caused by interactions of polyisoprene chains with non-isoprene compounds (proteins, lipids, *etc*.). This associative structure is gradually or partly destroyed when the polyisoprene is dissolved in conventional solvents (cyclohexane, toluene, tetrahydrofuran, *etc*.). Thus, in many cases, a proportion of the natural rubber remains insoluble in such solvents; this fraction is commonly called the gel phase or *macrogel* (also called apparent or soft gel), removed by centrifugation[1]. This gel phase, though complicated by the multitude of physical and chemical interactions that could contribute to maintaining the polymer's associative molecular structure, has been studied for its origin[2–4], its different forms[1] and its influence on the properties of the material[5–7]. This gel is often given additional emphasis during studies on storage hardening[8–10], a phenomenon that amplifies gel formation in natural rubber. The soluble fraction contains the polyisoprene macromolecules, and a variable quantity of micro-aggregates of 1 – 15 µm in diameter[11], forming the *microgel*, called real gel by some authors[1], that can be eliminated from the solution by filtration. Grechanovski

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et al.[12] showed that adding an alcoholic solution of acid or base in a hexane solution of NR eliminated the macrogel by reducing interactions with proteins. They concluded that acid or base treatment hydrolyzed bonds between polyisoprene chains and proteins. Tangpakdee and Tanaka[4] treated NR solutions in toluene with sodium methoxide (transesterification) and noticed a concomitant disappearance of NMR peaks of ester groups on polyisoprene chains with the elimination of macrogel. They proposed the presence of phospholipids at the polyisoprene chain terminals are involved in interactions responsible for the gel formation[3].

However, most of these studies, especially those undertaken by Tanaka *et al.*[4, 10,13,14], only took into account the macrogel while ignoring the microgel. It could have been thought that the macrogel was determined alone due to its relative ease of quantitation (by centrifugation) as compared to the microgel whose tedious quantitation only became much obvious with steric exclusion chromatography (SEC) as shown by Ngolemasango *et al.*[15]. But this was not the case as SEC was used during the course of some of the studies previously cited. In fact, the quantity of microgel as compared to that of the macrogel, is far from being negligible, and the quantities of these two entities are often negatively correlated[16], the more the macrogel content is in a NR sample the lesser the microgel content, and vice versa. It has even been demonstrated that during storage hardening, the microgel of a grade TSR 10 natural rubber (natural coagulation) is transformed to macrogel, without any significant change in the molar mass distribution[15].

In this study, we monitor the formation of microgel using SEC during a purification process, transesterification with sodium methoxide, as well as that of the whole rubber during the dissolution and purification processes. Indeed, it has even been shown that the elimination of esters on the polyisoprene chains had more dramatic effects on the structure of the NR than deproteinisation[4]. Additional analysis of the composition of phospholipids in monoclonal model NR samples were undertaken to clarify their role in NR's associative structure.

MATERIALS AND METHODS

Preparation of Rubber Samples

Latex was collected from two *Hevea brasiliensis* clones (PB 217 and GT 1) and processed to obtain NR samples of grades TSR 3CV and TSR 10. Each clone consisted of a homogeneous stand of about 150 trees planted in 1974, opened for tapping in 1980 and in regular tapping (every 4 days on half-spiral). The harvested latex was collected in cups and about half of the latex in each cup was retrieved and bulked to prepare grade TSR 3CV rubber and the remaining portion processed to grade TSR 10 rubber. To process grade TSR 3CV rubber, ammonia was added to preserve the fresh latex (1 L NH_4OH at 5% per 100 L latex) and to prevent premature coagulation. Neutral hydroxylamine sulphate (0.8 g/kg dry rubber) was introduced in the latex to stabilise the rubber by inhibiting branching between polyisoprene chains[2]. The latex was later coagulated at pH 5.0 using formic acid (0.15% v/v latex) and left to mature for 16 h. The coagulum was granulated, washed and dried for 4 h at 115ºC on an industrial processing line. For grade TSR 10 rubber, the latex was left to coagulate naturally in the cups. The coagula were retrieved after 3 days, deposited on barns for an additional 21 days, granulated, washed and dried for 5.5 h at 115ºC. These different drying times were a reflection of the duration required to give dry rubber of acceptable humidity.

The molar mass distributions of the processed rubber from the two clones were quite different with that of the GT 1 being bimodal and that of the PB 217 being unimodal (*Figure 1*).

Gel Content and Weight-average Molar Mass Determination

Cyclohexane (AnalaR), stabilised with 2,6-di-tert-butyl-4-methylphenol, was used to dissolve rubber test portions (2 mg/mL solvent) in tightly sealed jars, maintained at 30ºC for 14 days. Dissolution was accompanied by gentle stirring for 1 h each day after which the solutions were centrifuged (35,000 g for 1 h at 17ºC) and the macrogel (m_{MG}) quantified as the mass of the dried centrifugation residue.

The macrogel-free solution was diluted to 0.2 mg/mL, stored in the dark for 24 h and filtered through a 1 µm glass fibre. The eventual supernatant was injected into a size exclusion chromatograph (SEC) with two 20 µm PLGEL columns and a UV detector operating at 220 nm[16,17]. Calibration was carried out using synthetic poly(*cis*-1,4-isoprene) standards (Standards Kit No. WAT035708, Polymer Standards Service Inc., USA).

The microgel was considered as the portion eliminated by the 1 µm filtration[16] and determined from the polyisoprene peak area of the chromatogram. Using UV detection with SEC for a given chromatogram, the Beer-Lambert law applies ($A = \varepsilon\ l\ C$) as the area of the rubber peak is proportional to the concentration of the injected solution. "A" is the absorbance, "ε" the molar extinction coefficient, "l" the cell length (cm), and "C" the solution concentration (in mg/mL). For a given sample injected into the SEC apparatus, a calibration curve $S = f(C)$ should give the concentration of the solution after filtration (S is the area under the rubber peak and C the concentration of the injected solution). The calibration curve $S = f(C)$ was obtained from polyisoprene standards indicated above. Thus, knowing the concentration of the solution before and after filtration enables the quantification of the fraction eliminated by filtration or microgel content, as detailed in the Appendix.

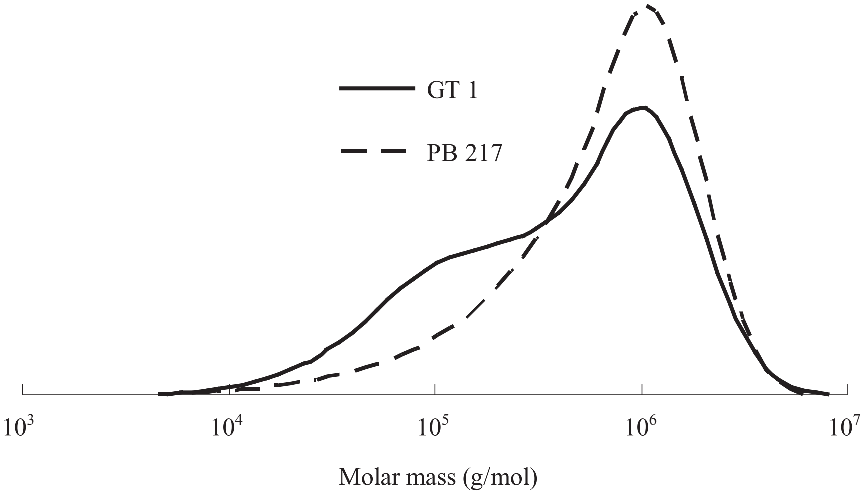


Figure 1. Initial molar mass distributions of the natural rubber samples of grade TSR 3CV from clones GT 1 and PB 217.

Purification by Transesterification of Rubber in Solution

Some NR was dissolved in AnalaR grade toluene (300 mg in 50 mL of solvent) which had been previously stabilised with 2,6-di-tert-butyl-4-methylphenol at 125 mg BHT/L solvent. 5 mL of 2 M sodium methoxide (33.3 mmoles of $NaOCH_3$/g rubber) was added to this solution and refluxed for 2.5 h in the dark. The solution was then cooled to room temperature and all excess reagents neutralised with 5 mL hydrochloric acid (1 M) in diethyl ether. The polyisoprene in solution was recovered by precipitation in 7.5 volumes of methanol and stored in a refrigerator overnight. The precipitate was dried under a hood for 48 h and *in vacuo* at 50ºC for 4 h, and then conserved in a desiccator for at least 30 min prior to analysis.

RESULTS AND DISCUSSION

Transesterification of Whole Rubber

Transesterification provoked more or less significant changes in the structure of the model rubber samples as the macrogel phase was always completely dissolved while the microgel was never completely dissolved (*Table 1*). Whereas transesterification did not change the microgel content of the TSR 10 from clone GT 1, those of the other samples were considerably dissolved: by about half for the TSR 10 of clone PB 217 (from 57 to 32%) and about a third for TSR 3CV of clone GT 1 (from 34 to 26%). Transesterification also caused a significant reduction of the number-average molar mass ($\overline{M}_n$) of the soluble phase of the clone GT 1 grades (20 to 26%) and almost no change (5%) on the TSR 10 of clone PB 217. In a similar manner, transesterification had a significant influence on the molar mass distributions (MMD) of the model grades (*Figure 2*), provoking the disappearance of some high molar mass chains and the appearance of low and average molar mass chains. However, as indicated in *Table 1*, the quantities of rubber injected in the SEC were very different for the control and transesterified samples. By weighting the proportion of rubber injected into the SEC (multiplying the proportion of the phase injected by the molar masses of the species involved), it was observed that transesterification released a much more significant quantity of low and average molar mass chains from samples of grade TSR 10 (*Figure 3*). It was equally observed that the MMD of clone PB 217 - TSR 10 rubber remained unimodal after transesterification while the bimodal nature of the MMD of clone GT 1 - TSR 10 rubber became more marked. This confirmed the presence of two populations of chains in clone GT 1 rubber characterised by an inherent bimodal MMD (MMD_0) and a single population in the clone PB 217 rubber

TABLE 1. EFFECT OF TRANSESTERIFICATION WITH SODIUM METHOXIDE ON THE MESOSTRUCTURE OF WHOLE NATURAL RUBBER GRADE TSR 10 FROM DIFFERENT CLONES

Clone	Treatment	Gel content (%, w/w rubber) Macrogel	Microgel	Gel total	Fraction injected in SEC (%)	Molar mass (kg/mol) $\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
GT 1	Control	41.2	26.5	67.7	32.3	768	154	5.1
	Transesterified	0.2	25.6	25.8	74.2	511	114	4.5
PB 217	Control	14.2	57.1	71.4	28.6	947	267	3.6
	Transesterified	0.6	31.5	32.1	67.9	785	253	3.1

of unimodal MMD_0. Although the sodium methoxide was largely in excess in the reaction medium (> 33 mmol g^{-1} rubber), increasing its concentration from 3.6 to 89.6 mmol g^{-1} rubber did not eliminate all the microgel as the latter attained a minimum when treated with about 15 to 20 mmol g^{-1} rubber (*Figure 4*). At the highest methoxide concentration tested, the macrogel was completely dissolved while ~ 30% of microgel persisted in solution.

Transesterification of Sol and Macrogel Fractions

Separate transesterification of the sol fraction (soluble phase) and the macrogel was conducted to check if the macrogel could release, in addition to the polyisoprene chains, some transesterification-insensitive microgel. These tests were conducted on grade TSR 10 rubber (of clone GT 1) with high macrogel content: 30% cyclohexane-insoluble or 7.2% toluene-insoluble. Each fraction was separately transesterified using the same procedure adopted for transesterification of whole rubber. After transesterification, the macrogel was almost totally dissolved releasing essentially microgel particles (95.7%) while the sol fraction gave mostly polyisoprene chains and a microgel content of about 17.6% (*Table 2*). These gel contents are expressed with respect to the quantity of rubber in the transesterified phase. We also expressed the macrogel and microgel contents with respect to the initial mass of whole NR put in solution and compared these with results presented in *Table 1*. Indeed, of the microgel insensitive to transesterification (~ 25% of initial material), about 30% originated from the macrogel while the remainder was microgel already present in the sol fraction (≈ 70%).

These results enable us to confirm the observations of Tangpakdee and Tanaka[4] who had demonstrated that transesterification of NR decomposes the macrogel and some high molar mass polyisoprene chains. However, part of the NR (25% to 30%, depending on the clone), not sensitive to transesterification,

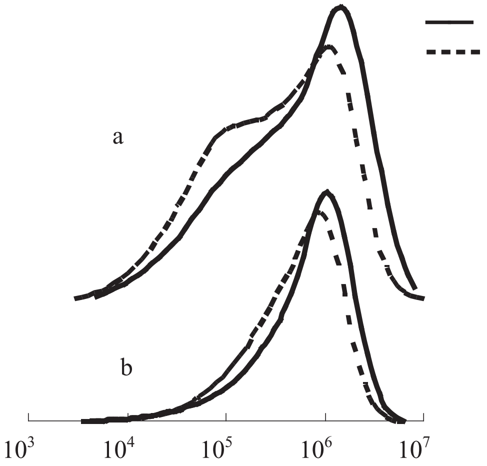


Figure 2. MMD of untreated and transesterified rubber of different origins: (a) TSR 10 of clone GT 1 and (b) TSR 10 of clone PB 217.

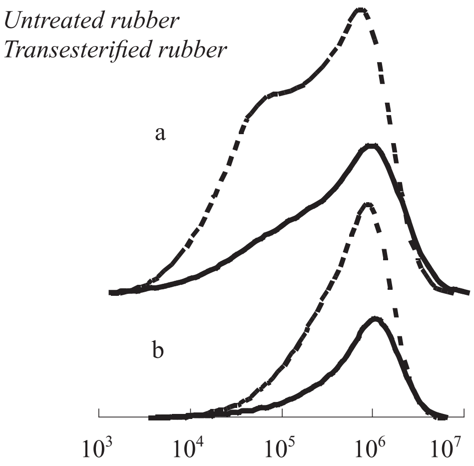


Figure 3. MMD of untreated and transesterified rubber after weighting by the % proportion of rubber injected for (a) TSR 10 of clone GT 1 and (b) TSR 10 of clone PB 217.

TABLE 2. EFFECT OF TRANSESTERIFICATION WITH SODIUM METHOXIDE ON THE STRUCTURE OF NATURAL RUBBER OF GRADE TSR 10 (GT 1)

Components	Whole rubber (%w/w)	In cyclohexane after transesterification Soluble phase (% w/w of) Test portion	 Initial rubber	 Macrogel phase (% w/w) Test portion	 Initial rubber
Macrogel	0.2	0.7	0.65	2.0	0.14
Microgel	25.6	17.6	16.3	93.7	6.7
Total gel	25.8	18.3	17.0	95.7	6.9
Injected in SEC	74.2	81.7	74.5	4.3	0.3

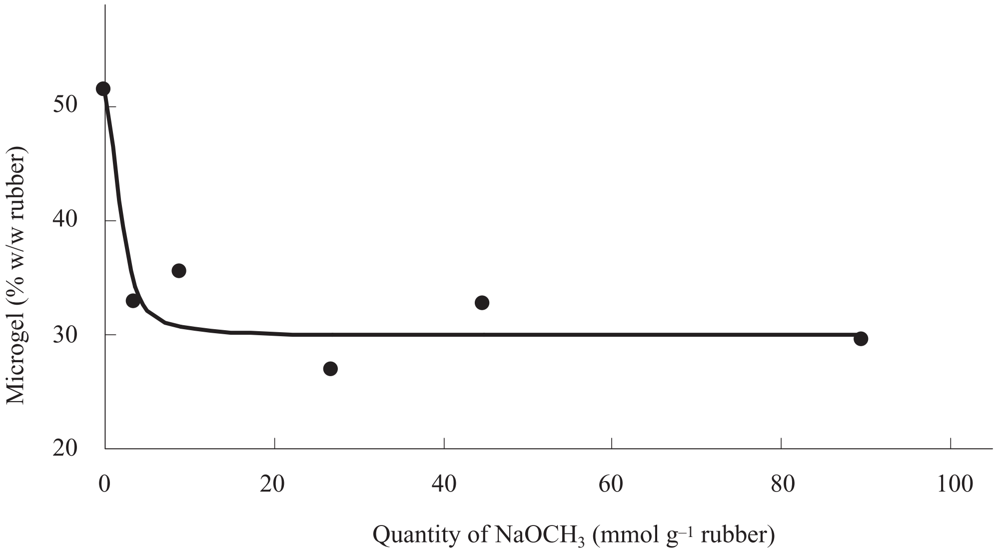
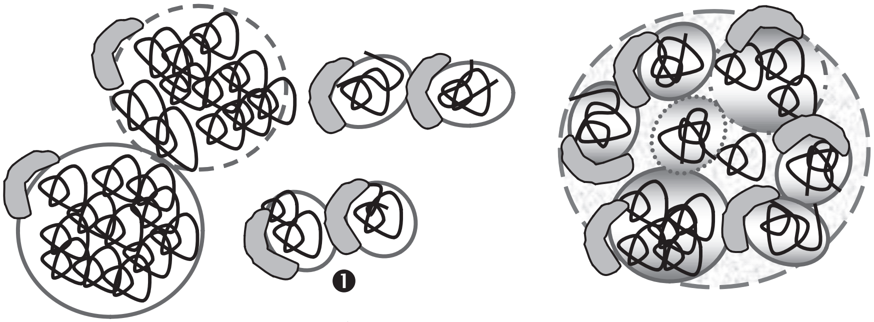
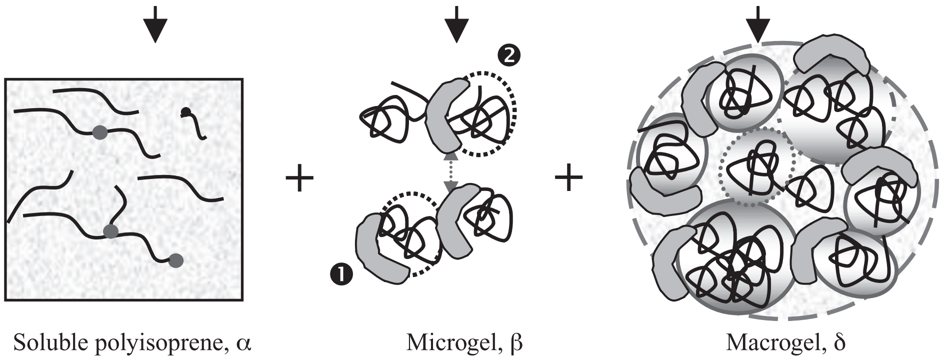


Figure 4. Evolution of the microgel content with the concentration of sodium methoxide during transesterification of whole natural rubber (grade TSR 10, clone GT 1).

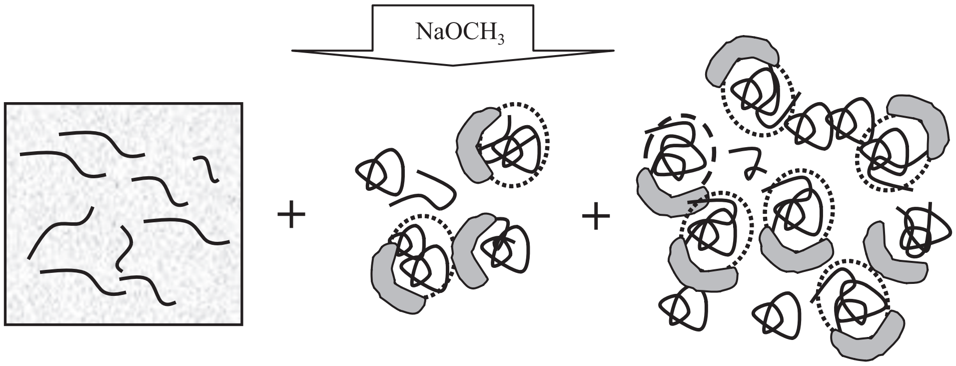
remained in the form of microgel. Our results also highlight the appearance of a large quantity of low and average molar mass chains, originating probably from macrogel and/or microgel, or part of the polyisoprene chains entrapped in the gel. Transesterification of the macrogel obtained following dissolution of rubber in toluene (macrogel that remained insoluble in toluene) released essentially microgel. On the basis of results reported here and those of the literature, the structure of the NR as well its evolution during dissolution and after transesterification could be schematised as in *Figure 5*. From this, it could be seen that the macrostructure of *Hevea brasiliensis* NR is made up essentially of entities with variable sizes (*Figure 5a*) which are more or less delimited by a phosphoglycolipoproteic membrane. On dissolution, this structure is denatured to give more or less branched polyisoprene chains – microgel and macrogel (*Figure 5b*). The proteins involved in



a) Macrostructure



b) Structure after dissolution



c) Structure after transesterification

❶ Proteins

❷ Microgel

More or less degraded membrane

←--→ Interaction with proteins

Figure 5. Schematic representation of the evolution of the structure of natural rubber following dissolution and transesterification.

biosynthesis and associated with the rubber membrane (Rubber Elongation Factor and/ or *cis* Prenyl transferase)[18,19] hinder the dissolution of polyisoprene in cyclohexane. Indeed, the magnitude of this phenomenon becomes greater and more significant as the sizes of the rubber particles get smaller. These entities will constitute the microgel and will contribute in the structuring of the macrogel. A second phenomenon which could be considered is the denaturing of the larger particles during processing from latex to solid rubber. This process would then contribute to associations between polar lipids and polyisoprene chain ends to generate high molar mass polyisoprene chains, which are later easily destroyed by transesterification (*Figures 5b* and *5c*). The macrogel, whose quantity varies with the polarity of solvent used, breaks up primarily into microgel (*Figure 5c*).

CONCLUSIONS

In organic solvents, *Hevea* natural rubber (NR) separates into a soluble fraction of long polyisoprene chains, micro-aggregates (microgel) and a dense insoluble macrogel. Previous studies on *Hevea* gel were devoted to macrogel (probably due to its relative ease of quantification), but advances in quantifying microgel using size exclusion chromatography (SEC) have facilitated further investigation into the gel phase and its contribution to the structure of NR.

The structure of the two model *Hevea* rubber grades (TSR 3CV and TSR 10) used for this study evolved following dissolution and transesterification. Transesterification completely converted the macrogel to generate some low-to-average molar mass chains as well as some microgel. The microgel, representing as much as one-third of the rubber's weight fraction, did not dissolve completely and often remained non-sensitive to transesterification. Transesterification also modified the molar mass distributions of the model grades, provoking the disappearance of some high molar mass chains and the appearance of low and average molar mass chains. Compensating for differences between untreated and transesterified samples for the quantities of soluble rubber injected in the SEC, did not only confirm the inherent unimodality and bimodality of some *Hevea* clones (indicating the presence of one or two populations of chains, respectively) but also signalled the generation of a much more substantial proportion of low and average molar mass chains from the macrogel.

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APPENDIX

Calculations leading to the quantitation of microgel content in Hevea natural rubber using size exclusion chromatography are presented here.

The microgel content was estimated as the percentage ratio of the mass of microgel ($m_{\mu G}$) to that of the rubber put in solution, m_0 (*Equation 1*):

$$\text{Microgel (\%)} = \frac{m_{\mu G}}{m_0} \times 100 \qquad \ldots 1$$

Meanwhile, the mass of the microgel ($m_{\mu G}$) was the product of the mass of the sol phase (m_{sol}) and the weight fraction of this phase (X_i, in %) that was eliminated by filtration (*Equation 1a*):

$$m_{\mu G} = m_{sol} * X_{inj} \qquad \ldots 1a$$

- The mass of the sol fraction,

$$m_{sol} = m_0 - m_{MG} \qquad \ldots 1b$$

- The fraction of sol eliminated by filtration, $X_{inj} = \{[C]_s - [C]_{inj}\}/[C]_s$ … 1c

m_{MG} was the mass of macrogel, $[C]_s$ and $[C]_{inj}$ represent respectively the concentration of the diluted sol phase and that of the diluted and filtered sol phase (for SEC injection).

The mass of microgel (*Equation 1a*) then becomes:

$$m_{\mu G} = (m_0 - m_{MG})^* ([C]_s - [C]_{inj})/[C]_s \quad \ldots 1d$$

The microgel content (%) according to *Equation 1* then becomes:

$$\text{Microgel (\%)} = \left(\frac{\dfrac{(m_0 - m_{MG})^*([C]_s - [C]_{inj})}{[C]_s}}{m_0} \right) \times 100$$

$$= \frac{(m_0 - m_{MG})^*}{m_0} \frac{([C]_s - [C]_{inj})}{[C]_s} \times 100$$

$$= (100 - MG)^*(1 - ([C]_{inj}/[C]_s)) \quad \ldots 1f$$

Considering that the concentration of the diluted sol phase $[C]_s$ is known and equal to 0.2 mg/mL, *Equation 1f* becomes:

$$\text{Microgel (\%)} = (100 - MG) * (1 - 5[C]_{inj}) \quad \ldots 2$$

Where MG was the macrogel content (in %) of the raw rubber and $[C]_{inj}$ the concentration of the solution injected after filtration, determined from the polyisoprene calibration graph S = f(C).

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