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AN ALTERNATIVE ROUTE TO PRODUCE STANDARDS FOR GEL PERMEATION CHROMATOGRAPHY USING NITROXIDE MEDIATED POLYMERIZATION

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Abstract - All over the world standards for Gel Permeation Chromatography (GPC) are produced using ionic polymerization. Standards are commercialized in a broad range of molecular weight and their dispersity (Ð) must be lower than 1.1. This work proposes the synthesis of polystyrene standards using Nitroxide Mediated Polymerization (NMP), an alternative technique to produce controlled polymers that is much more robust when compared to ionic polymerization. Standards with different ranges of molecular weights were obtained, all of them with very narrow molecular weight distribution (MWD) and dispersity (Ð) lower than 1.10. In order to do that, several combinations of different initiators were tested. Advanced GPC Triple Detector was used to obtain important properties, such as absolute number and weight average molecular weights, dispersity and intrinsic viscosity. The analytical method used in the characterization of the samples was in-house validated in terms of linearity, accuracy, precision, repeatability and robustness. The validation study demonstrated the quality of the measurements and ensured that the information obtained for a given analyte by the GPC technique is reliable.
Keywords: GPC polystyrene standard; Nitroxide mediated polymerization; Mixture of initiators.

INTRODUCTION

In the last two decades, reversible-deactivation radical polymerization, before called controlled radical polymerization or living radical polymerization, has been used to produce polymers with highly controlled microstructure. A polymer is considered controlled if it exhibits: a) a linear evolution of ln[1/(1-conversion)] versus time, (b) a linear increase of the number-average molar mass (Mn) versus conversion and (c) dispersity indexes (Ð) lower than 1.5. Research in this area usually follows one of the three techniques: Atom Transfer Radical Polymerization

(ATRP), Reversible Addition-Fragmentation Chain-Transfer (RAFT) and Nitroxide Mediated Polymerization (NMP). In all the three cases there is a reversible equilibrium during the whole polymerization between the active species and dormant species, reducing the concentration of free radicals and, consequently, minimizing the irreversible termination step.

Nitroxide Mediated Polymerization involves the addition of a controller or trapping agent (stable nitroxide radical) to capture the growing chain. Solomon *et al*. (1986) were the first researchers to use nitroxide radicals in free radical polymerization, but they synthesized only polymers with low molecular

weight. Georges *et al.* (1993) published one of the most cited papers related to NMP. After that, many studies have been conducted in the NMP area with different purposes such as to better understand the kinetic mechanism (Hawker *et al.*, 2001; Veregin *et al.*, 1996; Bonilla *et al.*, 2002), to enhance the rate and control the polymerization (Nabifar *et al.*, 2009; Wong *et al.*, 2011; Harrison *et al.*, 2012; Dias *et al.*, 2007), to produce copolymers (Hlalele and Klumperman, 2011; Alison *et al.*, 2014) and to functionalize polymers (Harrison *et al.*, 2011; Cai *et al.*, 2006; Ruehl *et al.*, 2008). Charleux *et al.* (2007) gave an important contribution using different kinds of controllers (SG1-based alkoxyamines), in order to make NMP possible at lower temperature. Although the dispersity obtained in all these studies was lower than 1.5, usually the values were higher than 1.1 and the focus of these works was not to produce controlled polymers in a broad range of molecular weight.

Nitroxide Mediated Polymerization is successfully applied to produce polystyrene and the polymer produced using this route is very pure. Since the most used polymer for standards in GPC is polystyrene and the standards must be very pure, this present work aims to develop polystyrene standards using Nitroxide Mediated Polymerization, a robust and innovative technique compared with ionic polymerization, currently used to obtain GPC standards. NMP is simpler and cheaper, no complicated purification steps are needed, and controlled radical reactions require less stringent conditions for impurities and temperature.

The high quality of standards in the GPC technique is very important to obtain reliable results, since the standards are necessary to build the calibration curves needed to acquire quantitative and qualitative data.

The calibration of a GPC column is performed by injecting polymer standards of known molecular weights and the calibration method can be performed in three different ways: i) *Conventional calibration*, in which only one concentration detector is used (refractive index or ultraviolet); ii) *Universal calibration*, in which two detectors are used (refractive index or ultraviolet and a viscosity-detector); and iii) *Triple detector calibration*, in which the integrated detectors used are refractive index or ultraviolet, viscosity detector and light scattering detector.

Conventional and universal calibrations require the use of at least 8 standards (between 8 and 12 standards), with different and clearly defined molecular weights, all of them with dispersity close to 1 (ranging between 1 and 1.10), in order to construct a calibration curve which is used as a reference for polymer analysis. In triple detector calibration a well characterized single standard is needed, with accurate information

of molecular weight, intrinsic viscosity and dispersity, in order to calibrate a few constants for each detector used.

The experiments reported in this paper used a mixture of initiators with different decomposition constants, in order to obtain polymers of different molecular weights. The initiators used in this work were TBEC (tert -butylperoxide -2-ethylhexyl carbonate), Trigonox C (tert-butylperoxy benzoate), Trigonox 101 (2,5-dimethyl-2,5-di(tert-butylperoxy) hexane) and Perkadox L-W75 (benzoyl peroxide). Mixtures of these initiators were also used.

The purity of the controlled polymers synthesized was confirmed by Fourier Transform Infrared Spectroscopy analysis.

A detailed validation in terms of Linearity, Accuracy, Precision, Repeatability and Robustness for the analytical method was performed, in order to guarantee that the information obtained from GPC is reliable.

MATERIALS AND METHODS

Polymerization Method

The synthesis of polystyrene was carried out in sealed glass ampoules. In this technique, the monomer must be purified of any inhibitor added by the manufacturer, so the styrene (Acros Organics, 99%) was washed three times with 10% NaOH solution followed by twice with deionized water. After that, the solution was dried over calcium chloride. The controller TEMPO (Acros Organics, 98%) and the initiators TBEC (Sigma-Aldrich, 95%), PERKADOX L-W75 (Akzo Nobel, 75%), TRIGONOX C (Akzo Nobel, 99%) and TRIGONOX 101 (Akzo Nobel, 92%) were used as received.

Desired aliquots of monomer, mixture of initiator and controller were weighed, mixed, and added into glass ampoules. The ampoules were sealed after removal of oxygen (inhibitor agent) by three successive freeze-thaw cycles under vacuum (0.03 mbar). Finally the ampoules were placed in an oil circulating bath heated at 135 °C to carry out the polymerizations.

After the polymerization time, the ampoules were weighed and broken. The polymer/monomer was removed from ampoules, dissolved in tetrahydrofuran (THF) and precipitated with ethanol.

The purification of the polymer starts with the evaporation of solvents and residual monomer, controller and initiators, followed by drying the sample in a vacuum oven heated at 60 °C to complete the purification of the polymer. After this step the samples were weighed for gravimetric analysis.

Polymer Characterization

GPC Analysis

The number and weight average molecular weight (Mn and Mw) and dispersity of the polymers obtained were characterized by GPC. The equipment used was a Viscotek TDA 302 chromatograph equipped with refractive index, right angle light scattering (RALLS) and intrinsic viscosity (IV-DP) detectors, equipped with two Viscogel I-series columns, 300 x 7.5 mm, particle size 10 μm and pores 10^6 and 10^3 (Viscotek). Two columns were used instead of three because, by comparison, we observed that two columns provided a good resolution, showing effective results for the commercial standard (Viscotek). The use of two columns instead of three minimizes time and cost of analysis, which is important for a commercial product. Tetrahydrofuran HPLC grade was used as mobile phase (40 °C) and sample eluent. The calibration was carried out using polystyrene standards for triple detector GPC calibration (Viscotek).

Infrared Analysis

The polymer was characterized by FT – Infrared Spectroscopy, measuring range 4000 – 450 cm^{-1}, on samples prepared using KBr plates. The equipment employed was a Spectrum One FT-IR Spectrometer (Perkin Elmer).

Validation of Analytical Methodology

In this work an in-house validation of the GPC method was also performed. Validation of analytical methodology for characterization of polystyrene samples was intended to verify the reliability of the analysis method, since such samples will be used as the standard in the GPC technique. This study evaluated the parameters linearity, accuracy, precision, repeatability and robustness.

The *Linearity* was evaluated from the linear regression of an analytical curve obtained by a series of 5 injections of solutions with different concentrations of a sample analyte, in this case a sample synthesized in this work. A calibration curve was built correlating signals from the refractive index detector and the concentrations of the solutions at each point. The correlation coefficient (r) of this straight line was used to evaluate the quality of the calibration curve.

In order to evaluate the *Accuracy* of the analytical method, the agreement between data obtained experimentally and the reference value (the one accepted as true) was verified. In order to obtain the experimental

data, injection in triplicate of 3 preparations of a standard sample was considered, since the GPC analysis usually requires 3 replicates of each sample.

The reference values were the nominal values of polystyrene standard (NIST - Traceable) informed by the manufacturer (Viscotek): Mn 5870, Mw 6220, Mz 6040, PDI 1.06 and IV 0.0698. Mean and standard deviation values obtained for each triplicate were compared to the nominal values.

The *Precision* parameter was evaluated by repeating injections of the same sample in order to verify the dispersion of Mn, Mw, Mz, Mp and PDI (measurement precision). The dispersion of the results obtained in different preparations (method precision) was also evaluated. For the precision test three preparations of a polymer sample were performed with concentrations 1.5; 3.5 and 5.5 mg / ml.

According to Ribani *et al.* (2004), the precision parameter is evaluated by the calculation of the absolute standard deviation, which uses a significant number of samples (more than 20). However, the number of samples needed for the validations methods is usually small, and the validation can be performed using 9 samples (3 levels of concentration in triplicates).

In the injection *Repeatability* study, values of retention volume and standard deviations (%) were evaluated for injections in triplicate of a commercial standard sample (Viscotek, Mw 6040 and PDI 1.06) at a concentration of 3 mg/ml.

A method is considered robust when it is not affected by a slight modification of its parameters such as concentration of the organic solvent, pH, ionic strength of the mobile phase in liquid chromatography and temperature. The *Robustness* was analyzed by injecting in triplicate samples obtained in this work at concentrations 1.5; 3.5 and 5.5 mg / ml.

RESULTS AND DISCUSSION

The first section below summarizes all syntheses performed in order to obtain polymers suitable to be used as GPC standard. Different initiators and mixture of initiators are used, as well as different operating conditions (concentration of initiator and ratio between controller and initiator), in order to obtain polymer with different values of molecular weights and dispersity lower than 1.10. We then verified if the purity degree obtained in the synthesized polymers was equivalent to that of a commercial standard. Finally we evaluate the synthesized samples in the GPC calibration method and validate the analytical method in terms of Linearity, Accuracy, Precision, Repeatability

and Robustness, in order to guarantee that the information obtained from GPC is reliable.

Synthesizing Different Polymers to Be Used as GPC Standards

Table 1 shows the initiators used in this work as well as their characteristics. All experimental conditions used in this work are summarized in Tables 2 and 3.

Table 1: Molecular weight, activation energy (Ea), pre-exponential factor (k_{do}) of the initiators.

Initiator (I)	Molecular Weight (g/mol)	Ea (kJ/mol)	k_{do} (s^{-1})
TBEC	246.40	131.86	6.98×10^{-8}
PERKADOX LW75	242.25	122.35	6.47×10^{-5}
TRIGONOX C	194.23	151.59	3.04×10^{-5}
TRIGONOX 101	290.40	152.69	3.50×10^{-9}

(Data Akzo Nobel Company)

Runs 1, 2 and 3 (Table 2) were performed using only one initiator. In runs 1 and 2, it was chosen to work with TBEC as initiator, because it allows obtaining controlled polymers with faster reactions when compared to PERKADOX as initiator (Nogueira *et al.* (2010)). PERKADOX used in run 3 is able to provide polymer with very low dispersity; however, when it is used, the polymerization rate is much slower than the one obtained with initiators with lower decomposition rate constants (k_d), like TBEC, for example. In this work mixtures of initiators (see Table 3) with different decomposition constants (k_d) were also analyzed.

All polymerization reactions were performed at 135 °C. It is well known that NMP using TEMPO requires high temperature (between 125 – 145 °C). The temperature 135 °C was chosen based on the previous study conducted in our research group (Nogueira *et al.*, 2010), regarding the best operating temperature to maintain the highest polymerization rates for the NMR process using TBEC or PERKADOX-LW75 as initiators.

Table 2: Polymerization conditions using TEMPO as controller.

Run	Initiator (I)	[I] (mol/L)	R*
1	TBEC	0.036	1.3
2	TBEC	0.036	1.6
3	PERKADOX	0.036	1.6

* R = [controller]/[initiator]

Table 3: Polymerization conditions using TEMPO as controller, where RI=[I$_1$]:[I$_2$] is the initiator ratio, [I$_T$] is the total concentration of initiator in the reaction, and R=TEMPO]:[I] is the [controller]/[initiator] ratio.

Run	Mixture of Initiator	RI	[I$_T$] (mol/L)	R
4	TBEC+PERKADOX	1:1	0.050	1.6
5	TBEC+PERKADOX	1:1	0.036	1.6
6	TBEC+PERKADOX	1:1	0.018	1.6
7	TBEC+PERKADOX	1:1	0.009	1.6
8	TBEC+PERKADOX	1:1	0.009	2.0
9	PERKADOX + TRIGONOX.C	1:1	0.036	1.6
10	TBEC + PERKADOX + TRIGONOX. 101	1:1:1	0.036	1.6

Effect of [TEMPO]/[I] Molar Ratio

Figure 1 (a), (b) and (c) show conversion, number average molecular weight and dispersity profiles for runs 1 and 2, in order to evaluate the effect of [TEMPO]/[TBEC] ratio on the dispersity values. It was possible to verify that keeping the concentration of TBEC (0.036 M) and increasing the [TEMPO]/[TBEC] ratio from 1.3 to 1.6, the induction time increases (Figure 1a); however, much lower dispersity values were obtained (Figure 1c).

These results are in accord with the kinetic model studied by Veregin *et al.* (1996), where the authors reported that an increase in the initial concentration of TEMPO leads to a decrease in dispersity. The dispersity values obtained in a NMP process are inversely proportional to the exchange rate between the active species by the release and capture of the controller during the polymerization.

Results show that the use of high [TEMPO]/[I] molar ratio, greater than 1.3, generates a reduction of dispersity, which is essential for the production of GPC standards. In this way the ratio [TEMPO]/[I] used in the next experiments was 1.6.

## Effect of Mixture of Initiators in the Polymerization Reactions

In order to analyze the effect of using more than one initiator, Figure 2 shows run 5 (TBEC+ PERKADOX) compared with run 2 (TBEC) and run 3 (PERKADOX). All runs have the same operating conditions: [I] = 0.036 M, [TEMPO]/[I] ratio = 1.6 and T = 135 °C. It can be seen that TBEC initiator, which presents a low decomposition rate, shows the highest values of dispersity even having the smallest size of

polymeric chains. When using PERKADOX (which has faster decomposition rate compared to TBEC) pure or in combination with TBEC, the reaction rate becomes slower; however, higher molecular weight is obtained, keeping low values for dispersity. For the mixture of initiators (run 5), dispersity lower than 1.10 is observed even at high conversion. The molecular weight versus conversion profiles for runs 3 and 5 showed very similar behavior.

In Figure 2a, it was expected that the polymerization rate for run 5 (that uses the mixture TBEC + PERKADOX) would be greater than the one for the run 3 (PERKADOX), since the lower k_d of TBEC makes controlled polymerization faster. However, the polymerization rate for run 5 was similar to the one for run 3. This suggests that the polymerization rate was mainly influenced by the initiator that has the higher k_d value, in this case, PERKADOX.
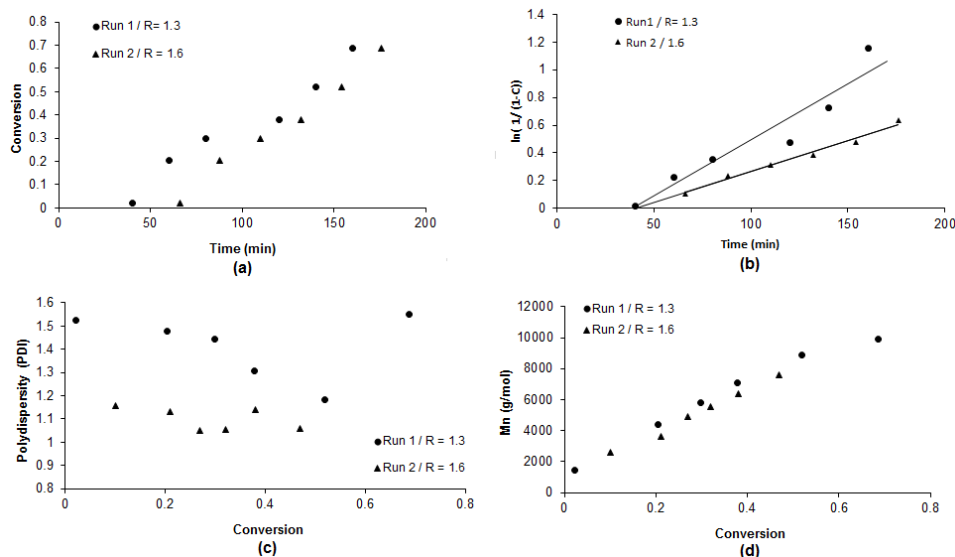
Figure 1: Effect of [TEMPO]/[TBEC] ratio for runs 1 and 2 using TBEC at 135 °C. (a) conversion versus time, (b) ln(1/(1-C)) versus time, (**c**) dispersity versus conversion, and (d) molecular weight versus conversion.
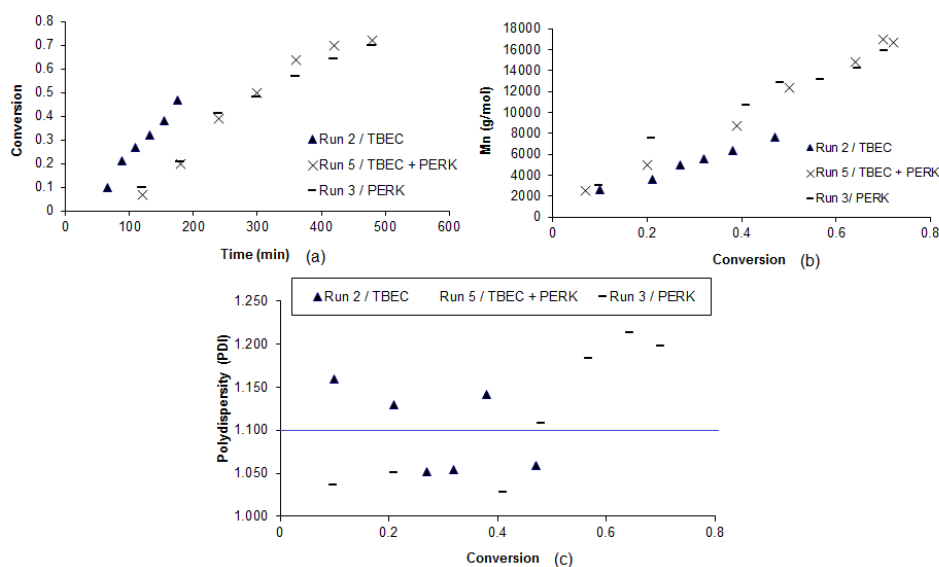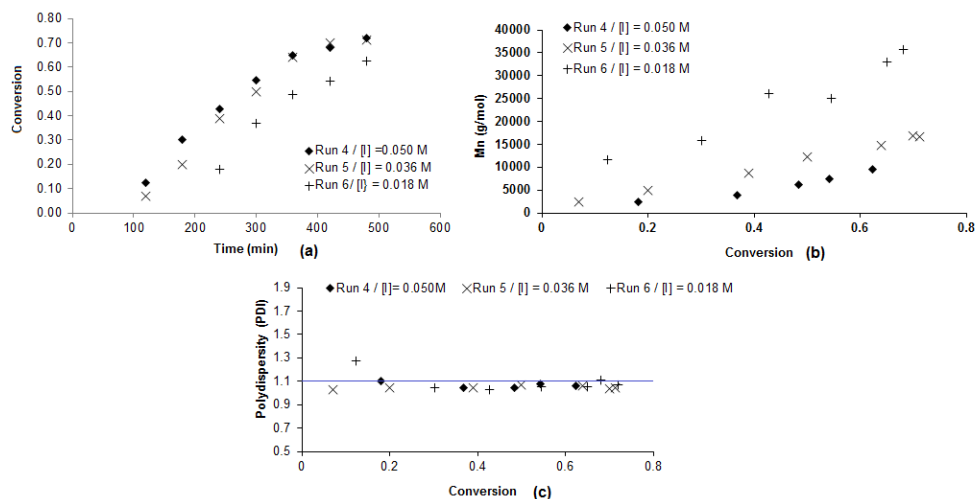

Figure 2: Effect of the type of initiator on (a) conversion versus time, (b) M_n versus conversion and (c) polydispersity versus conversion profiles for runs 1, 3 and 5 using the [TEMPO]/[I] molar ratio = 1.6 at T = 135 °C.

Figure 3 shows the conversion, number average molecular weight and dispersity profiles for runs 4, 5 and 6, where the mixture of initiators TBEC+PERKA-DOX was used at concentrations 0.050, 0.036 and 0.018 M, respectively. For all three cases the mixture of initiators promoted a very low dispersity, ranging between 1.03 and 1.11, independent of the concentration of initiator used. The molecular weight profiles increase linearly with the conversion for the three cases, which represents a good control of the polymerization process. It can also be seen that higher concentration of initiator promotes a lower molecular weight, since the monomer has to be incorporated in a bigger amount of growing chains.

From the results obtained, it is possible to see that the mixture of initiator has a great potential to produce GPC standards, because polymers with dispersity lower than 1.1 of different molecular weights can be produced.

In order to obtain polymers with molecular weight ever higher, the concentration of initiator was reduced to 0.009 mol/L (runs 7 and 8). Figure 4 shows that, keeping the [TEMPO]/[I] molar ratio equal 1.6, polymers with molecular weight up to 56,000 g/mol were obtained. Roa-Luna *et al*. (2007) used different TEMPO/BPO molar ratios (0.9, 1.1, 1.2 and 1.5) to produce controlled polystyrene, and also observed that high molecular weights are obtained when the controller/initiator ratio decreases.

Figure 3: Effect of mixture of initiator and TEMPO concentration on (a) conversion versus time, (b) M_n versus conversion and (c) polydispersity versus conversion profiles for runs 4, 5 and 6 using the [TEMPO]/[I] molar ratio = 1.6 at T = 135 °C.
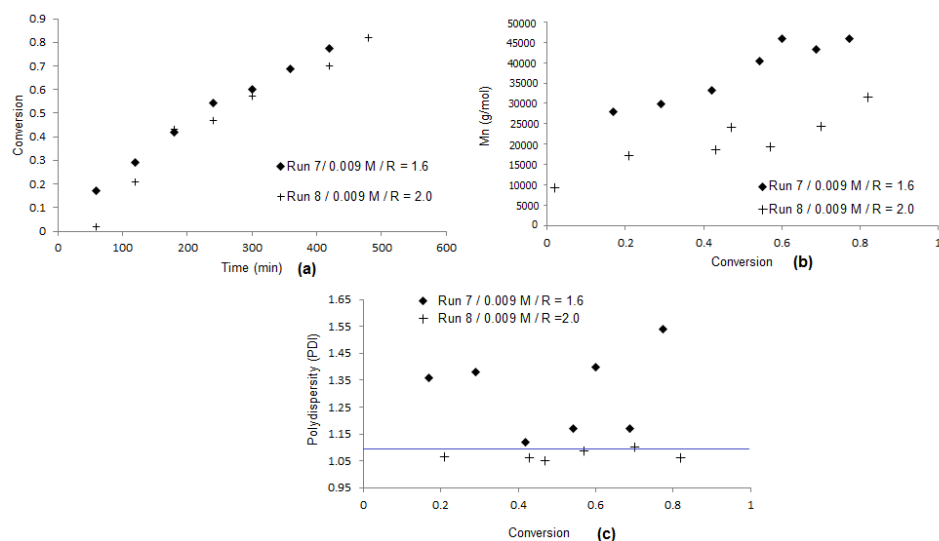
Figure 4: Effect of the ratio between TEMPO and initiator concentration on (a) conversion versus time, (b) M_n versus conversion and (c) dispersity versus conversion profiles (runs 7 and 8) using the [TEMPO]/[I] molar ratio = 1.6 at T = 135 °C.

Run 7 demonstrated a lower dispersity compared to conventional polymerization, because most of the dispersity values are very close to the limit value (1.5). However, the Mn versus conversion profile is not typical for a controlled polymerization, indicating an inadequate control for a GPC standard. The polymers obtained in runs 7 and 8 were not used as GPC standards.

In order to analyze different kinds of mixtures of initiators, runs 5, 9 and 10 were taken into account, as described in Table 3. In all cases the same final concentration of initiator (0.036M) and [TEMPO]/[I] molar ratio (1.6) were used. Figure 5 displays the results.

It was found that mixtures of different types of initiator were able to produce polymers with dispersity smaller than 1.10 for all the three cases.

Results show that mixtures of initiators PERKADOX + TRIGONOX C and PERKADOX + TBEC + TRIGONOX 101 are also effective alternatives to obtain controlled structure polymer to be applied as GPC

standard, because Mn can vary depending on the mixture of initiator used, always keeping dispersity lower than 1.10.

Evaluation of Polymer Purity by Infrared Spectroscopy

Fourier transform infrared spectroscopy (FT-IR) analysis was performed for a commercial polystyrene (American Polymer) and for a sample synthesized in this work. Through the spectra it was possible to evaluate if the NMP method was effective to obtain polystyrene with the purity degree needed for a commercial standard.

Table 4 summarizes the absorption frequency ranges corresponding to the functional groups for the polystyrene obtained in this work and for the polystyrene of a commercial standard. Figure 6 displays the overlay of FT-IR spectra.
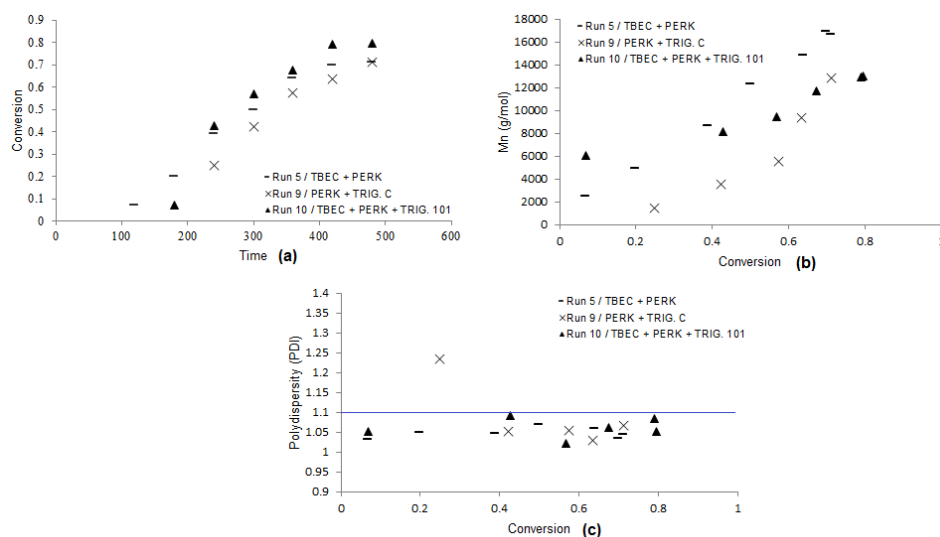

Figure 5: Effect of different types of mixtures of initiator on (a) conversion versus time, (b) M_n versus conversion and (c) polydispersity versus conversion profiles for runs 5, 9 and 10 using the [I] = 0.036 M, [TEMPO]/[I] molar ratio = 1.6 at T = 135 °C.

Table 4: FT-IR absorption frequency ranges corresponding to the functional groups for polystyrene.

Bond	Functional Group	Absorption Frequency range (cm^{-1})	Commercial Standard (cm^{-1})	Synthesized Sample (cm^{-1})
-CH-	alkenes, aromatics	3100 – 3000	3030	3025
-CH-	aliphatic alkanes	2960 – 2850	2885	2849
-C=C-	Aromatics	1600 – 1450	1495	1492
-CH$_2$-		1470 – 1430	1455	1452
-CH-	Mono subst out of plane bending	770 - 730 ou 710 - 690	765	756
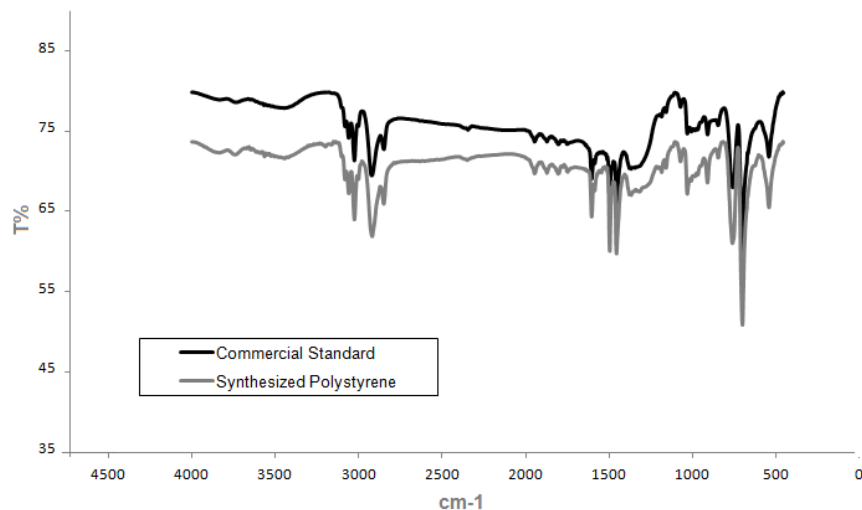
Figure 6: FT-IR overlay spectra for commercial standard and synthesized polystyrene.

The overlay of FT-IR spectrum of the commercial and synthesized samples confirms that the experimental method used in this work to synthesize the polymer is effective, since the band positions for both samples show good agreement.

Evaluation of Synthesized Samples in the GPC Calibration Method

First of all a Conventional Calibration Curve was obtained using samples of different runs and different degrees of conversion. The samples chosen to build the Conventional Calibration Curve are shown in Table 5 and Figure 7.

The calibration curve was obtained using Omnisec 4.1 (Viscotek) software and a TDA 302 Triple Detector Chromatograph, where only the refraction index detector was used.

The experimental points were fitted to a polynomial of degree five (or a quintic function) and the linear region was the part of the curve used to measure the molecular weight of samples. It was also observed that the linear range of the calibration curve had a linear coefficient (r^2) equal 0.995, which is a satisfactory result, since the curve should have a value equal or greater than 0.950.

Figure 8 shows a polystyrene sample analyzed with the conventional calibration curve obtained (Figure 7).

In order to verify the effectiveness of the calibration curve, an analysis of a polystyrene sample with predefined values of molecular weight, viscosity and dispersity was performed considering both conventional and triple detector calibration. Results are compared in Tables 6 and 7.

Triple detection calibration used refractive index, viscosity and light scattering detectors and the commercial standard Viscotek 115 K for triple detector (NIST-Traceble). The Viscotek 115 K standard nominal values informed by the manufacturer were: Mn = 113 g/mol, Mw = 114 g/mol, Mz = 115 g/mol, dispersity = 1.009 and IV = 0.516 dL/g.

Table 5: Synthesized polymers to be applied on conventional calibration curve.

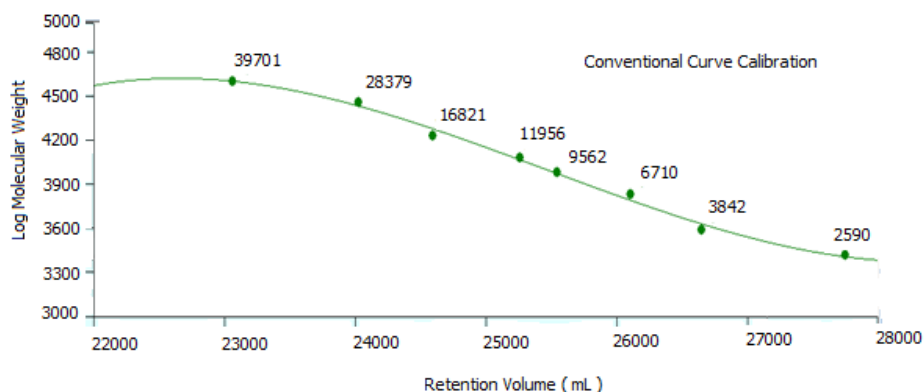| Synthesized Polystyrene	Mn (g/mol)	Mw (g/mol)	Mp (g/mol)	Đ(Mw/Mn)	IV (dl/g)
1	2352	2582	2590	1.098	0.0498
2	3842	4008	3842	1.043	0.0553
3	6105	6359	6710	1.042	0.0720
4	8808	9275	9562	1.053	0.0954
5	11568	12043	11956	1.041	0.1050
6	15875	16344	16821	1.030	0.1246
7	28468	26624	28379	1.069	0.1863
8	33038	36598	39701	1.100	0.2093

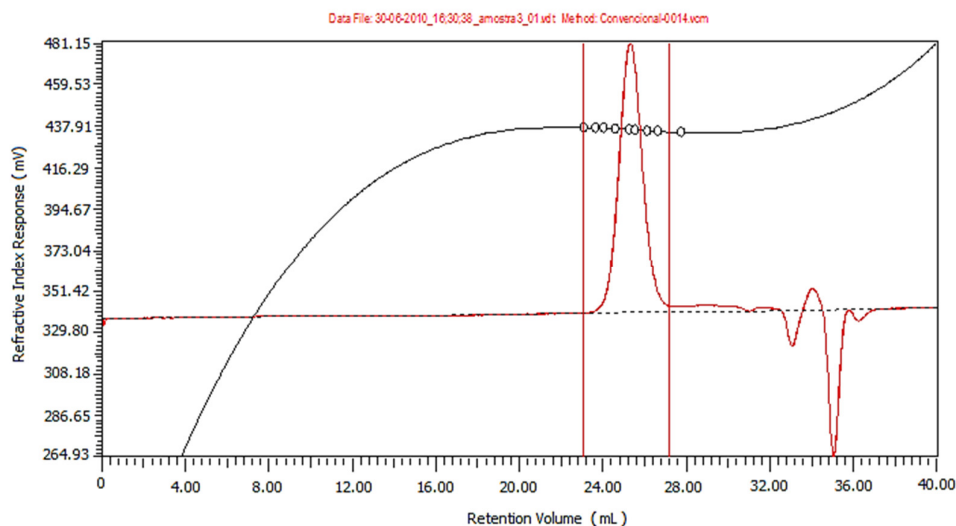**Figure 7:** Conventional calibration curve.

Figure 8: Chromatography profile of a conventional calibration.

The commercial standard and synthesized samples were compared by the response of analysis of a specific sample in conventional and triple detector calibrations. The objective was to determine if the synthesized samples used in conventional calibration were capable to show the same response that a commercial standard used in triple detector calibration shows.

It was observed that the responses obtained by the triple detector calibration are the same or very close to the ones obtained by the conventional calibration that uses the standards produced in this work. These results show that the standards produced were very effective for the GPC calibration, since the Triple Detection calibration is known to be an effective and precise method that uses one single commercial standard to calibrate a few constants for each detector used.

The relative errors *er* (%) of each measurement were less than 10 %, which is an expected value for GPC analysis, and shows that the samples produced, when applied in conventional calibration, were able to

respond very well compared to a Triple Detection calibration.

Table 6: Mn, Mw, Mz, Mp and PDI results obtained for a sample in a conventional calibration (homopolymers).

Peak RV (ml)	Mn (Daltons)	Mw (Daltons)	Mz (Daltons)	Mp (Daltons)	Mw/Mn
26,803	3,748	4,296	5,011	4,073	1.146

Table 7: Mn, Mw, Mz, Mp and PDI results obtained for a sample in a triple detector calibration (homopolymers).

Peak RV (ml)	Mn (Daltons)	Mw (Daltons)	Mz (Daltons)	Mp (Daltons)	Mw/Mn
26,803	3,460	4,126	4,657	4,252	1.539

It is important to observe that controlled polymers produced in this work could be applied as standards

for samples with molecular weight lower than 40,000 g/mol if Conventional or Universal calibration methods are used. However, any controlled polymer produced in this work (with dispersity lower than 1.1) could be used as standard for samples with any molecular weight if Triple Detector calibration is considered. This is because the Triple Detector calibration only needs standards with dispersity lower than 1.1 and well defined values of Mw, Mz and Mp and IV.

Validation of the Analytical Method

The validation of the analytical method was done in terms of Linearity, Accuracy, Precision, Repeatability and Robustness.

Linearity: It was observed that the refractive index signals vary in a linear way with the concentration values of the eight different samples, with correlation coefficient (r^2) equal to 0.9989.

Accuracy: It was observed that the method showed relative error values ranging from 0.0093 to 3.5945% for each sample injected in triplicate. Such accuracy percentage values are considered satisfactory for GPC analysis, because they should not exceed 10%.

Precision: The relative standard deviation (%) calculated for the results of the injections in triplicate demonstrated that they are very close, with values ranging from 0.033 to 0.7950. This results validate the precision parameter of the instrument due to consecutive injections of the same sample, since deviations in the GPC analyses are expected to be lower than 3%. Also the relative standard deviation for the arithmetic mean did not exceed 3%, proving the method has precision.

Repeatability: In the injection repeatability study, the retention volume values and the standard deviations (%) were evaluated for triplicate injections of a commercial standard sample (Viscotek – Mp 6040 and PDI 1.06). For each of the triplicate injections the retention volume (mL) was 16.93, 16.91 and 16.89, and the standard deviation was 0.1183, which demonstrated that the method has good repeatability.

Robustness: The method can be considered robust, because it was not affected by changes in concentration of the sample.

CONCLUSION

Although the NMP technique has long been studied in the controlled polymerization area, until the moment, to the best of our knowledge, this technique has not been used to produce standards for GPC, such

standards being synthesized by ionic polymerization.

In this study polymers ranging from 2000 to 40000 g/mol with dispersity values lower than 1.10 were produced using different mixtures of initiators and TEMPO as controller. Regarding the conventional and universal calibrations, the standards synthesized in this work could be applied to samples with molecular weight lower than 40000 g/mol. However, the standards synthesized in this work could certainly be used to analyze samples of any molecular weight if a Triple Detector calibration is considered, since this method needs only a single standard with well defined values of molecular weight, dispersity and intrisic viscosity.

The FT-IR analysis for commercial and synthesized polymer confirmed that the experimental method used in this work is effective, since the band positions for both samples showed a good agreement.

The GPC method was in-house developed and validated and showed that the polymers analyzed by this technique could certainly be used as standards, since the validation of the linearity, accuracy, precision, repeatability and robustness parameters confirms that it is reliable.

NMP using a mixture of initiators proved to be a good alternative to produce GPC standards, because it is a simple and low cost method, requires little space, no complex and expensive equipment is needed, and the reagents are commercially available.

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