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Two Dimensional Chromatographic Characterization of Block Copolymers of 2-Ethylhexyl Acrylate and Methyl Acrylate, P2EHA-*b*-PMA, produced via RAFT-Mediated Polymerization in Organic Dispersion

Jacques-Antoine Raust,*,[†] Lisa Houillot,[‡] Maud Save,[‡,§] Bernadette Charleux,[‡,∥] Claudine Moire,[⊥] Céline Farcet,[⊥] and Harald Pasch[#]

[†]*Deutsches Kunststoff-Institut (German Institute for Polymers), Schlossgartenstrasse 6, Darmstadt, Germany,* [‡]*UPMC Université Paris 6 and CNRS, UMR 7610, Laboratoire de Chimie des Polymères, 4 place Jussieu, 75252 Paris cedex 05, France,* [§]*IPREM Equipe Physique et Chimie des Polymères, UMR 5254, CNRS- Université de Pau et des Pays de l'Adour, Technopole Hélioparc, 2 Av Président Angot, 64053 PAU cedex 9, France,* [∥]*Université de Lyon, Univ. Lyon 1, CPE Lyon, CNRS UMR 5265, Laboratoire de Chimie Catalyse Polymères et Procédés (C2P2), Equipe LCPP Bat 308F, 43 Bd du 11 novembre 1918, F-69616 Villeurbanne, France,* [⊥]*L'Oréal Recherche, 1 avenue Eugène Schueller, 93600 Aulnay sous Bois, France, and* [#]*SASOL Chair of Analytical Polymer Science, Department of Chemistry and Polymer Science, University of Stellenbosch, Private Bag X1, 7602 Matieland, South Africa*

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ABSTRACT: For the precise characterization of block copolymers of 2-ethylhexyl acrylate (2EHA) and methyl acrylate (MA) produced via RAFT (reversible addition−fragmentation chain transfer)-mediated dispersion polymerization, novel liquid chromatographic separations have been developed. SEC showed multimodal molar mass distributions (MMD) and HPLC showed multimodal chemical composition distributions (CCD). The analyses of MMD and CCD of the reaction products indicated the formation of the expected block copolymer along with remaining P2EHA and PMA homopolymer fractions. Online coupling of SEC and gradient HPLC in a two-dimensional liquid chromatography (2D-LC) setup proved to be an efficient method to fractionate all polymer species present in the samples. Different kinds of copolymer molecules were identified in addition to the two homopolymers. The quantification of P2EHA using liquid chromatography at critical conditions (LC−CC) showed that the unreacted macro(RAFT agent) amount remained unchanged during at least the first 4 h of polymerization. LC−CC experiments also allowed the relative molar mass of the PMA blocks contained in the copolymers to be determined. The implementation of 2D-LC combining SEC and LC−CC allowed a more precise characterization of the different copolymer structures in particular in terms of block size. Finally, the results obtained by SEC/HPLC were confirmed by LC−^{1}H NMR (proton nuclear magnetic resonance) experiments. It was concluded that the dispersed state of the polymerization system was the important factor for the formation of broadly distributed, complex copolymers when using a dithiobenzoate-based reactive macromolecular stabilizer. The detailed characterization of the system highlighted the enhancement of irreversible termination at the interface of the dispersed particles.

Introduction

High-performance liquid chromatographic (HPLC) techniques are best suited for analyzing polymer heterogeneity.[1,2] Size exclusion chromatography (SEC) is the established method for analyzing polymer molar mass distribution, while interaction chromatography (e.g., isocratic or gradient HPLC, or liquid chromatography at critical conditions: LC−CC) is the preferred technique for analyzing chemical heterogeneity.[3,4]

Frequently, gradient HPLC is used for chemical composition separation since it enables mobile phase composition to be adjusted to maximum selectivity and problems related to polymer solubility to be overcome. In gradient HPLC, precipitation and adsorption on the stationary phase can occur and elution according to chemical composition is achieved by progressively increasing the elution strength of the mobile phase (solvent gradient).[5−7] An overview of different techniques and applications for chemical composition separation involving the combination of SEC and gradient HPLC was previously published.[1,2] Retention processes were discussed by Snyder et al. and they suggested different tests to identify the actual operative mechanism.[8,9]

A very peculiar mode of HPLC, specific for polymer analysis, is LC−CC. It is characterized by very narrow chromatographic conditions (stationary phase, mobile phase composition and temperature) which create a specific environment where enthalpic and entropic interactions between a given homogeneous polymer sequence (containing only one kind of repeat unit) and the chromatographic system exactly compensate each other. This results in an elution of these polymer sequences independent of their chain length (i.e., molar mass). For a homopolymer, elution of all macromolecules occurs at the system hold-up volume as if molar mass distribution was "invisible" for the system. In the case of diblock copolymers, the critical conditions of adsorption can be established for one block. The second block may then elute in the exclusion mode if repulsed from stationary phase or in the

*Corresponding author. E-mail: jacques-antoine.raust@evonik.com.

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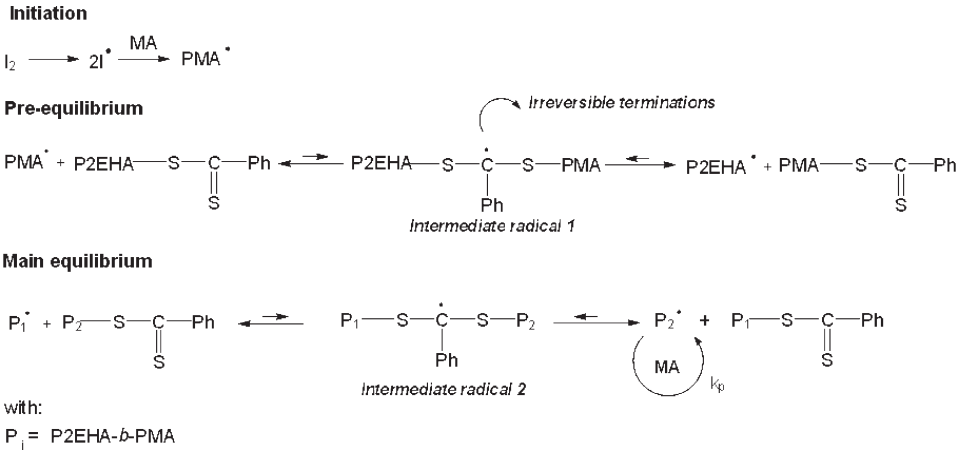
Scheme 1. Main Equilibrium Involved in Reversible Addition−Fragmentation Transfer (RAFT) Polymerization



adsorption mode if it has a higher affinity to the column packing. This peculiarity makes LC−CC a very attractive method for characterization of block copolymers.[10,11] Recent theoretical studies described precisely the influencing parameters,[12,13] and a review summarized critical conditions for a large number of analyzed polymers.[14] A method has been published to determine critical conditions for homopolymers with only few gradient HPLC experiments.[15]

The development of online two-dimensional liquid chromatography (2D-LC) allowed us to simultaneously recover information on different aspects of macromolecular heterogeneity. A fully automated two-dimensional chromatographic system including two chromatographs was introduced by Kilz et al.[16] who also published a review on the application of the technique to deformulate complex macromolecular structures.[17] In the first step, separation occurred by chemical composition using interaction chromatography and in the second dimension macromolecules were eluted as a function of their decreasing hydrodynamic volumes using SEC. Each fraction collected from the first chromatographic system was automatically transferred into the second separation system for SEC analysis. This kind of coupling is up to now the most common. Graft copolymers of methyl methacrylate and EPDM rubber were analyzed by Siewing et al. using 2D-LC (gradient HPLC coupled with SEC)[18] and Gerber and Radke[19,20] used 2D-LC to separate linear and star-shaped polystyrenes. Other examples of 2D-LC systems were published to characterize functional acrylate polymers, by Jiang et al.,[21] and complex acrylate−methacrylate copolymers for cosmetic applications, by Raust et al.[22]

A number of two-dimensional applications have been developed including the combination of LC−CC and SEC to analyze functional homopolymers, block copolymers and graft copolymers[23,24] or for the characterization of linear and star-block copolymers synthesized by ATRP.[10]

The aim of this work was to characterize molar mass and chemical composition distributions (i.e., MMD and CCD, respectively) of polyacrylate polymers produced via RAFT-mediated dispersion polymerization of methyl acrylate (MA) in isododecane (see Scheme 1) in the presence of dithiobenzoate capped poly(2-ethylhexyl acrylate) (P2EHA-DTB) macromolecular RAFT agent. This study follows previous works[25,26] in which we showed that when using *tert*-butyl dithiobenzoate as the chain transfer agent for the synthesis of the P2EHA block (i) the MA dispersion copolymerization allowed small particles (D < 100 nm) to be produced in situ in the dispersant isododecane medium, (ii) under some synthetic conditions these particles were polydisperse, and (iii) regarding the polymerization features, strong rate retardation and very poor control over the polymer structure were observed together with an incomplete consumption of the macro(RAFT agent) (P2EHA-DTB). Such results were quite surprising and in contradiction with the high efficiency of the P2EHA-DTB when used as a macro(RAFT agent) for polymerization performed either in bulk or in solution. Only a major influence of the dispersed state of the system could explain the difference in apparent reactivity of the macro(RAFT agent) used in this work. Different analytical techniques were then developed to characterize the copolymer formed and to better understand the synthesis process. In particular, we focused on the very first stage of the polymerization, at low MA conversion, when the system started to deviate from a well-controlled polymerization. A hypothesis of the polymerization mechanism to explain the formation of byproduct will be presented.

Experimental Section

Samples. The samples were synthesized via RAFT-mediated dispersion polymerization of methyl acrylate (MA) in isododecane at 80 °C, using dithiobenzoate-ended poly(2-ethylhexyl acrylate) (P2EHA-DTB) as a soluble macromolecular reversible chain transfer agent.[25−27] The reaction was initiated with *tert*-butyl peroxy-2-ethylhexanoate (T21S), and the RAFT agent used for the synthesis of P2EHA-DTB was *tert*-butyl dithiobenzoate. The experimental conditions are described in detail in our previous article.[25]

We analyzed samples which were taken in the first hours of a 20 h synthesis performed under the above-described conditions. Samples were taken after 1, 2, 4, and 8 h. Because of a long induction period, those samples showed low MA conversion: 3% after 1 h; 6% after 2 h; 11% after 4 h; 25% after 8 h. They were particularly interesting as the loss of control appeared very early in the reaction.

All samples were dissolved in THF for the analyses as it is a good solvent for both blocks and, therefore, fully dissolves them. This precaution was important to carry out chromatography on polymer chains and not on particles or micelles.

Chromatographic Equipment. Separations were carried out on a Shimadzu system comprising a DGU-14A degasser, a FCV-10ALvp solvent mixing chamber, a LC-10ADvp pump, and a SL 10ACvp auto sampler. For detection, an evaporative light scattering detector, ELSD 1000 (Polymer Laboratories, Church Stretton, England) was used. For data collection and processing, the software package "WinGPC-Unity" software v. 7.0 (Polymer Standards Service GmbH, Mainz, Germany) was used. For two-dimensional chromatography, sample fractions from the first dimension were transferred to the second dimension column via an electronically controlled eight-port valve system (type EHC8W, VICI Valco instruments, Houston, Texas, USA), with two 100 μL loops. The second dimension consisted of a Shimadzu LC-10ATvp pump.

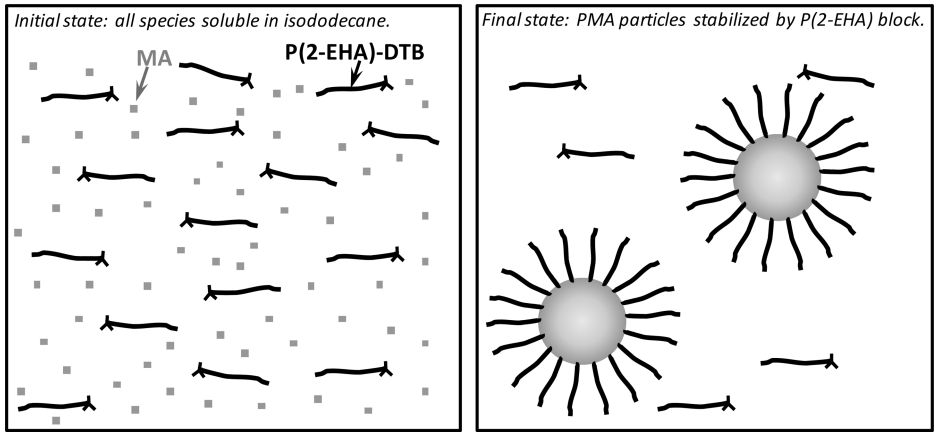


Figure 1. General representation of the P2EHA-DTB-mediated dispersion polymerization of MA.

A HPLC system Agilent 1100 consisting of a degasser, a quaternary gradient pump and an auto sampler, was used for the SEC-NMR measurements. The volume injected was 50 μL.

SEC. A set of three columns comprising PSS SDV (cross-linked styrene−divinylbenzene) with average pore sizes of 10^3, 10^4 and 10^5 Å, column sizes of 300 × 8 mm I.D. (Polymer Standards Service, Mainz, Germany), was used for the one-dimensional experiments and as first dimension in SEC × LC−CC 2D-LC experiments.

A PL HTS-C column with a size of 150 × 7.5 mm I.D. (Polymer Laboratories, Church Stretton, England) was used for the second dimension of gradient HPLC × SEC 2D-LC experiments.

All these separations were achieved with freshly distilled tetrahydrofuran (THF) as mobile phase using flow rates of 1.0, 0.04, and 1.5 mL/min for one-dimensional, SEC × LC−CC and gradient HPLC × SEC experiments, respectively.

In the case of the SEC-NMR experiments another set of three columns was used: PSS SDV 10^4, 10^5 Å, column size 50 × 20 mm i.d. (Polymer Standards Service, Mainz, Germany) and PL gel mixed-D column size 300 × 8 mm i.d. (Polymer Laboratories, Church Stretton, England) but using chloroform as the mobile phase. The volume injected was 50 μL. The flow rate was reduced to 0.5 mL/min to allow us to obtain a longer accumulation for NMR analysis.

Molar mass calibrations were conducted with a set of eight PMMA standards from PSS (Polymer Standards Service, Mainz, Germany).

All samples and standards were dissolved in THF in order to obtain polymer concentrations between 1 and 3 mg/mL.

HPLC and LC−CC. For adsorption chromatography and chromatography at critical conditions, columns of PLRP-S with 5 and 8 μm average particle sizes, respectively, column size 150 × 4.6 mm I.D. each (Polymer Laboratories, Church Stretton, England) were used. For gradient HPLC, a linearly changing binary mobile phase with a constant flow rate of 1 mL/min starting from methanol/THF (100/0 by volume) to methanol/THF (30/70 by volume) in 10 min was employed. LC−CC experiments were performed isocratically with a mobile phase composed of acetonitrile/THF (46/54 by volume) at 25 °C.

All samples and standards were dissolved in THF in order to obtain polymer concentrations between 5 and 10 mg/mL. For two-dimensional chromatography experiments the samples were more concentrated (between 5 and 20 mg/mL)

Solvents. Acetonitrile (ACN), methanol (MeOH) and chloroform ($CHCl_3$) were HPLC grade. Tetrahydrofuran (THF) was freshly distilled.

NMR. NMR experiments were performed on a 400 MHz spectrometer AVANCE (Bruker Biospin GmbH, Rheinstetten, Germany). The measurements were performed with a flow probe containing a 60 μL flow cell. The probe was a ^{1}H {^{13}C} inverse detection probe equipped with a shielded pulsed field-gradient coil. The gradient strength was 53 G/cm. The 90° ^{1}H pulse was 4.6 μs. WET solvent suppression[28] was applied to $CHCl_3$. One frequency was suppressed. Sixteen scans per free induction decay (FID) were acquired with an acquisition time of 1.1 s (16 K data points) and a relaxation delay of 0.1 s.

All samples and standards were dissolved in chloroform in order to obtain polymer concentrations around 10 mg/mL.

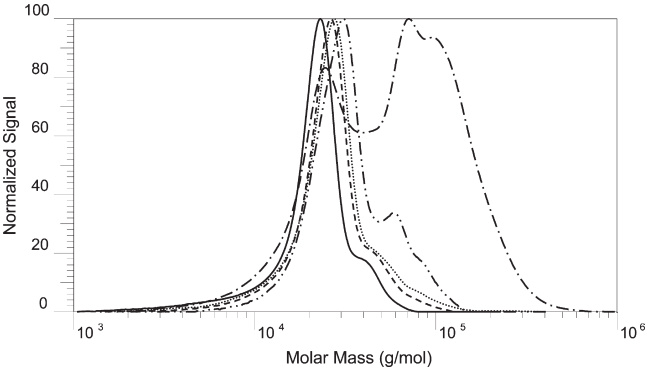


Figure 2. Relative molar mass distribution overlay of the P2EHA-DTB macro(RAFT agent) (solid line) with the four copolymers formed at various times: 1 h (dashed line), 2 h (dotted line), 4 h (double dot-dashed line), and 8 h (single dot-dashed line). Columns: PSS SDV 10^3, 10^4, 10^5 Å. Mobile phase: THF at 1 mL/min. Detection: RI. Calibration: PMMA.

Results and Discussion

To facilitate the explanations of the chromatographic results, a general scheme of the dispersion polymerization is presented in Figure 1. The RAFT-mediated dispersion polymerization of methyl acrylate in isododecane was carried out using dissolved poly(2-ethylhexyl acrylate) macro(RAFT agent), containing a dithiobenzoate reactive function. Isododecane being a nonsolvent for poly(methyl acrylate), the method produced stable colloidal particles, stabilized by the P2EHA soluble chains.

Analysis of MMD Using SEC. SEC analyses enabled the relative molar mass distribution of the samples taken at various reaction times to be evaluated. The chromatogram overlay of the macro(RAFT agent) with the four copolymer samples is shown in Figure 2. The average relative molar masses of these five samples are summarized in Table 1.

The relative molar mass overlay shows a shift of the peak maximum in the direction of higher molar masses when the polymerization time increases. According to the polydispersity values, one can assume that the radical polymerization

Table 1. Average Molar Masses Obtained by SEC for the P2EHA-DTB Macro(RAFT agent) and the Four Copolymer Samples (Calibration with PMMA)

sample	MA conversion (%)	$\overline{M_n}$ (g/mol)	$\overline{M_w}$ (g/mol)	PDI = $\overline{M_w}/\overline{M_n}$
P2EHA-DTB		19 500	23 500	1.20
COPO-1h	3	23 000	28 000	1.22
COPO-2h	6	23 500	30 000	1.28
COPO-4h	11	29 000	37 500	1.29
COPO-8h	25	37 000	76 000	2.05

remained controlled except for the last sample which is bimodal and as a result has a polydispersity index larger than 2. As can be seen in the SEC relative molar mass overlay, for the three other copolymers a shoulder at the higher molar mass side of the peaks appears. This shoulder is also present in the macro(RAFT agent). The shoulder is particularly noticeable for the sample taken at 4 h. This might indicate that control was lost during the reaction and especially between 2 h and 4 h as the shoulder tends to increase with reaction time. It has also to be noticed that the relative molar mass determined for the shoulder is roughly twice the value at peak maximum. The shoulder is most probably due to terminated polymer chains formed via coupling of two macroradicals.

For the sample at 8 h, the chromatogram indicates that macro(RAFT agent) is still present in the polymer forming the low molar mass peak. The poor symmetry of the high molar mass peak could indicate that several kinds of binary copolymer molecules were produced during the polymerization suggesting that control of the polymerization was partially lost. This is confirmed by the high value of PDI for this sample. For the first three samples taken between 1 and 4 h, the peaks of the copolymers still mostly overlay with the peak of the macro(RAFT agent), suggesting again that some nonreinitiated macro(RAFT agent) chains remained present. Because of the low resolution of SEC, this hypothesis was, however, not proven. Therefore, to characterize the copolymers according to their chemical composition, gradient HPLC and LC−CC experiments were performed on all samples.

Analysis of CCD Using Gradient HPLC. Gradient liquid adsorption chromatography (HPLC) allows for polymers to be separated according to their chemical composition. With this technique it should be possible to distinguish block copolymer molecules from remaining macro(RAFT agent) and PMA homopolymer species. The discrimination of the various macromolecules in the samples is based on polarity and solubility differences. In the present case, PMA is more polar than P2EHA. Both can be dissolved in methanol−THF, however, PMA is soluble in methanol−THF mixtures with lower amounts of THF as compared to P2EHA.

The separation of the species with different polarity was carried out on a nonpolar column packing material composed of cross-linked polystyrene−divinylbenzene. To enhance adsorption on this stationary phase, it was conditioned with methanol as a poor solvent of the polymer. As a result adsorption and/or precipitation of the macromolecules on the column material was achieved. The selective elution of the macromolecules according to their chemical composition was performed by linearly increasing the proportion of THF (good solvent) in the mobile phase. First, the more polar macromolecules containing higher proportions of methyl acrylate eluted. These fractions were followed by macromolecules containing an increasing proportion of 2-ethylhexyl acrylate. The expected order of elution is then: PMA followed by P2EHA-*b*-PMA and finally P2EHA.

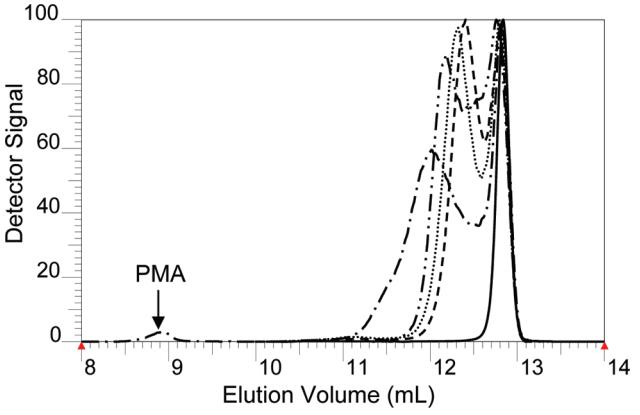


Figure 3. Gradient HPLC chromatogram overlay of P2EHA (solid line), COPO-1h (dashed line), COPO-2h (dotted line), COPO-4h (double dot-dashed line), and COPO-8h (single dot-dashed line). Columns: 2 PLRP-S 8 μm. Mobile phase: 10 min linear gradient from 0 to 70% of THF in methanol. Flow rate: 1 mL/min. Detection: ELSD.

Figure 3 shows an overlay of the chromatograms of the macro(RAFT agent) with those of the four copolymer samples analyzed by gradient HPLC. A uniform peak can be seen in the chromatogram for the P2EHA macro(RAFT agent) homopolymer at an elution volume of 12.8 mL. The narrow Gaussian form of the peak is characteristic of a homopolymer. On the contrary, the chromatograms of the other samples exhibit two peaks and even a third peak for the sample taken at 8 h. By analogy, the last eluted peak in all cases can be attributed to nonreinitiated macro(RAFT agent). Therefore, it shows that initiation by the macro-(RAFT agent) is incomplete.

The other peaks appear at lower elution volumes indicating that the detected macromolecules are more polar than P2EHA. The peaks are not completely separated from the macro(RAFT agent). They are ascribed to block copolymer fractions containing a polar block of PMA. The peak maxima shift toward lower elution volumes with increasing polymerization times, i.e., from 12.4 mL for COPO-1h to 12.0 mL for COPO-8h. This is the result of the growth of the PMA block.

Finally a peak eluting at V_e = 8.9 mL is detected for sample COPO-8h which corresponds to the elution volume of PMA. Apparently part of the polymerization occurred without the control by the RAFT agent and led to formation of free homopolymer chains of the second monomer.

For the samples taken at 1 and 2 h, the copolymer peak remained symmetric and Gaussian. This reveals that the chemical composition distribution was still well-controlled. However, it has to be noted that after 4 h a peak deformation appeared. This is likely to be a consequence of a loss of control. This tendency is confirmed by the chromatogram of COPO-8h: the copolymer peak maximum intensity decreases with polymerization time and the peak becomes broader. It remains difficult to define the nature of those chains, which are responsible for the peak deformation. Further experiments such as 2D-LC were necessary to get more detailed information.

To study the copolymers more in detail and to analyze the distribution of reactive chains with DTB end group, the same gradient HPLC separations were conducted using a UV detector in addition to ELSD. The detection was performed at a wavelength of 300 nm, which is specific for the DTB moiety. Therefore, the detector response is assumed to be exclusively due to DTB since polyacrylates do not exhibit absorptions at such wavelength. The gradient HPLC chromatogram overlay for COPO-2h obtained with both detectors is presented in Figure 4.

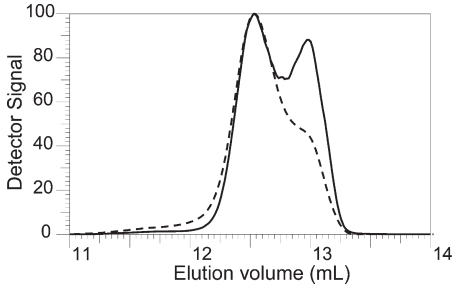


Figure 4. Gradient HPLC chromatogram overlay of sample COPO-2h. Columns: 2 PLRP-S 8 μm. Mobile phase: 10 min linear gradient from 0 to 70% of THF in methanol. Flow rate: 1 mL/min. Detection: UV 300 nm (dashed line) and ELSD (solid line).

As can be seen, there is a very good overlap of both signals for the copolymer peak at V_e = 12.5 mL. However, for the nonreinitiated P2EHA (V_e = 13.2 mL), the UV signal is weaker than that of ELSD. This would mean that, if all copolymer chains contain the DTB end group, only part of the P2EHA chains has one. These nonfunctionalized homopolymer chains are thus unable to undergo chain extension.

Characterization of Each Block with LC−CC. As baseline separation of the block copolymers and the residual macro-(RAFT agent) was not achieved, another chromatographic system was developed that operates at critical conditions for the P2EHA block. In such system, the P2EHA block is chromatographically "invisible" and all P2EHA homopolymer molecules elute at the same volume independently of chain length. Furthermore, the mobile phase was designed in such a way that it was a good solvent for the PMA block. Therefore, the PMA block was excluded from the stationary phase and, thus, separation of the copolymers was only driven by the PMA block length. In other terms, the separation corresponded to a SEC-like experiment only based on the PMA block.

In this part, the work focused on the three samples taken during the first 4 h of the polymerization process. The results of these experiments are shown in Figure 5 as an overlay of the chromatograms from the first three kinetic samples and the macro(RAFT agent).

The macro(RAFT agent) peak elutes at 3.27 mL which corresponds to the hold-up volume of the system. This is in agreement with the statement that the polymer eluting at critical conditions is "invisible" for the stationary phase. A comparison of Figure 3 and Figure 5 shows that a better separation of the copolymers and the residual macro(RAFT agent) is obtained by LC−CC as compared to gradient HPLC. The copolymer fractions elute before the hold-up volume of the column indicating that elution took place in the size exclusion region. As expected, with increasing polymerization time the copolymer peak maxima shift toward lower elution volumes, i.e., larger hydrodynamic volumes. This is caused by the growing PMA block length upon polymerization.

It should be noticed that the copolymer peaks for the samples taken during the first 4 h of synthesis are symmetrical. Molar mass resolution in the present case is much lower than that of the SEC experiment. This is the reason why the shoulders observed in Figure 2 are not seen in Figure 5. These copolymer peaks become broader with polymerization time indicating that the distribution of PMA block lengths increases during the synthesis. This observation was already made when the PDI values determined from SEC measurements were compared (see Table 1).

(*a*). *Quantification of Residual P2EHA*. The precise quantification of the nonreinitiated macro(RAFT agent) is possible by

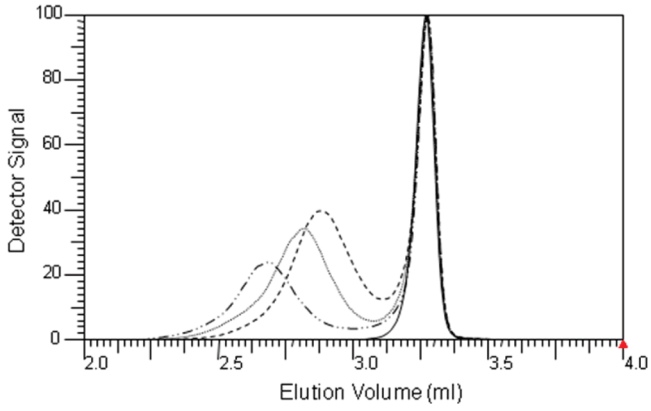


Figure 5. Chromatogram overlay of (solid line) P2EHA, (dashed line) COPO-1h, (dotted line) COPO-2h, and (dot-dashed line) COPO-4h at critical conditions of P2EHA. Stationary phase: 2 × PLRP-S. Mobile phase: ACN−THF 46:54% by volume; flow rate 1 mL/min; 25 °C. Detection: ELSD.

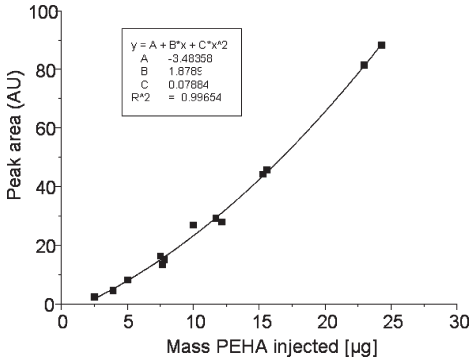


Figure 6. Calibration curve of ELSD detector response (peak area) vs injected mass of P2EHA macro(RAFT agent). Experimental conditions: see Figure 5.

LC−CC when ELSD is calibrated properly. The calibration curve for P2EHA (i.e., P2EHA peak area (at V_e = 3.27 mL) vs mass of P2EHA injected) is shown in Figure 6. As expected for ELSD calibration, the best fit was achieved with a second order polynomial function. According to these results the fit was sufficiently good to quantify residual macro(RAFT agent) in the copolymer samples.

The measurement of P2EHA peak area for three copolymer samples allowed us to calculate the ratio of residual macro(RAFT agent) mass to total injected mass of polymer (see fourth column of Table 2). From the ^{1}H NMR analysis of the samples, we were able to estimate the molar and mass proportions of 2EHA in the copolymers. With these two values, we estimated the percentage of P2HEA macro-(RAFT agent) chains which had not been reinitiated. The results are summarized in Table 2.

As expected for a progressing polymerization, the amount of 2EHA monomer in the copolymer decreased with synthesis time from 82.5 to 70.5 mol %. These values were converted into mass percentage using the molar masses of MA (86 g/mol) and that of 2EHA (184 g/mol). From LC−CC measurements, we observed that the mass percentage of nonreinitiated macro(RAFT agent) in the total injected polymer decreased with reaction time from 38.4 to 34.7 mass %. This result is expected as the addition of MA repeat units to the polymer chains increase the total sample relative molar mass. Combining these two values allowed us to estimate the percentage of 2EHA contained in the nonreinitiated macro(RAFT agent) chains from the total 2EHA

Table 2. Estimation of the Amount of Non-Reinitiated P2EHA Macro(RAFT agent) from ^{1}H NMR Measurements and Mass Percentage of Non-Reinitiated Chains

samples	total 2EHA in copolymer from ^{1}H NMR (mol %)	total 2EHA in copolymer from ^{1}H NMR (mass %)	nonreinitiated macro(RAFT agent) in total polymer from LC−CC (mass %)	percentage of total 2EHA contained in nonreinitiated macro(RAFT agent) (mass %)
COPO-1h	82.5	90.9	38.4	42.2
COPO-2h	79.0	88.6	35.9	40.5
COPO-4h	70.5	83.2	34.7	41.7

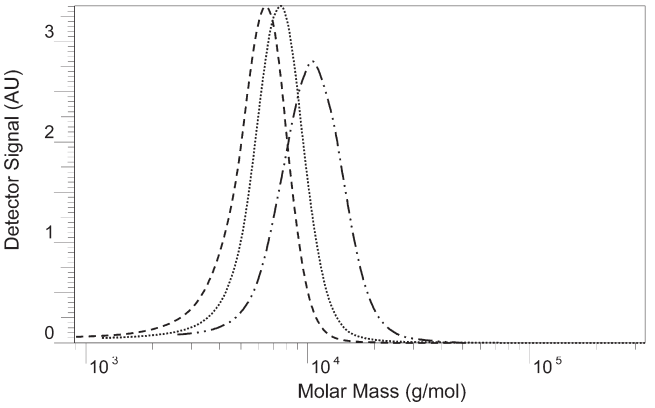


Figure 7. Copolymer peak relative molar mass overlay of COPO-1h (dashed line), COPO-2h (dotted line), COPO-4h and (dot-dashed line) at critical conditions of P2EHA. Experimental conditions see Figure 5. Calibration: PMMA.

contained in the injected sample. The comparison of the values given in the fifth column of Table 2 shows that this amount remained nearly constant during the whole polymerization: around 41.5%. This means that approximately 59% of the macro(RAFT agent) chains were reactivated during the first hour of polymerization (MA conversion = 3%) and that nonreinitiated chains remained inactive until the end of the reaction.

Bathfield et al. found similar results when polymerizing *n*-butyl acrylate in dispersion with poly(*N*-acryloylmorpholine) as macro(RAFT agent).[29] They analyzed the supernatant of the latex after centrifugation using ^{1}H NMR and found that only 55% of the introduced dithiobenzoate-capped macro(RAFT agent) was indeed involved in the formation of the particles. Their measurement was done after 89% conversion. This makes of course a difference with the low conversion (< 10%) for COPO-4h, but it was seen that the quantity of nonreinitiated macro(RAFT agent) remained constant during the first 4 h of polymerization and should remain so during the whole process.

(*b*). *Determination of MMD for the PMA block*. As mentioned before, LC−CC for P2EHA separates copolymers only according to PMA block length, assuming that the P2EHA block is "invisible" for the chromatographic system. This specificity was used to determine the average molar masses of the PMA blocks after calibrating the system with PMMA standards. PMMA is more polar than P2EHA and elutes similar to the PMA block in the exclusion mode.

Figure 7 shows an overlay of copolymer peaks for the first three samples with a molar mass calibrated *X*-axis. Table 3 summarizes the average relative molar masses determined with this method. These values refer only to the PMA block as the copolymer chains eluted irrespective of the P2EHA block. The results confirmed the relative molar mass increase of the PMA block with monomer conversion and showed rather low polydispersity indices.

2D-LC Gradient HPLC × SEC: Combination of CCD and MMD Information. 2D-LC experiments where gradient HPLC was coupled to SEC gives more comprehensive results

Table 3. Average relative molar masses of PMA blocks in binary copolymers, LC−CC experiments at critical conditions for P2EHA

	$\overline{M_n}$ (g/mol)[a]	$\overline{M_w}$ (g/mol)[a]	PDI = $\overline{M_w}/\overline{M_n}$
MA block of COPO-1h	4500	5800	1.29
MA block of COPO-2h	6200	7900	1.27
MA block of COPO-4h	8800	12000	1.36

[a] Molar masses obtained by LC−CC after calibration of the system with PMMA standards.

for the analyzed samples. The 2D-LC contour diagrams for the macro(RAFT agent) and the four polymer samples are shown in Figure 8. The SEC information appears on the *X*-axis and gradient HPLC information on the *Y*-axis. The red lines indicate the peak integration limits.

Following details have to be taken into account to better comprehend the results of 2D-LC experiments. The results of the 2D-LC integration in the article are given in relative peak volumes (percentage) which actually do not represent the mass percentage of the species in the total polymer, since each kind of (co)polymer gives a different response with the ELSD detector. A quantitative approach would necessitate the calibration of the ELSD with standards for each kind of (co)polymers, which are obviously not available. The color scale in 2D-LC characterizes the height of the spots, whereas the relative peak volumes help to consider the importance of each species. This is particularly important for Figure 8E. In this 2D-LC plot, the spot 5 appears as a minor peak according to the color scale but it in fact represents one of the major products of the polymer. In this article the 2D-LC results are mainly used as a qualitative tool to try to understand the different reactions, which occur during the RAFT synthesis.

The relative volumes and average relative molar masses for each peak are given in Table 4 and the proposed macromolecular architectures for all peaks are given in Table 5. It has to be noticed that all average relative molar mass estimations determined from 2D-LC experiments are very close to those given by 1D-SEC measurements.

Figure 8A shows the 2D-LC plot of the macro(RAFT agent). As can be seen, it is very narrowly distributed along the *Y*-axis corresponding to chemical composition. This is an expected result for a homopolymer. However, along the *X*-axis, a deviation can be seen at the high molar mass side in the direction of higher elution volumes. This deviation can be related to the shoulder at the high molar mass side of the chromatogram detected in the SEC experiment of the macro-(RAFT agent) (black curve in Figure 2). It is also observed in the 2D-LC plots and particularly for sample COPO-4h as a separate spot (see peak 3, Figure 8D). This peak presumably corresponds to macromolecules formed by irreversible termination reactions of P2EHA growing chains. One hypothesis could be formulated to explain the observed separation: the absence of the DTB end group for the byproduct and the doubled relative molar mass lead to a stronger adsorption on the stationary phase and thus a slightly delayed elution in gradient HPLC. The use of the UV detector was inefficient for these 2D-LC experiments as the polymer concentration is very low and the coupling products represent only a few percent of the total polymer sample.

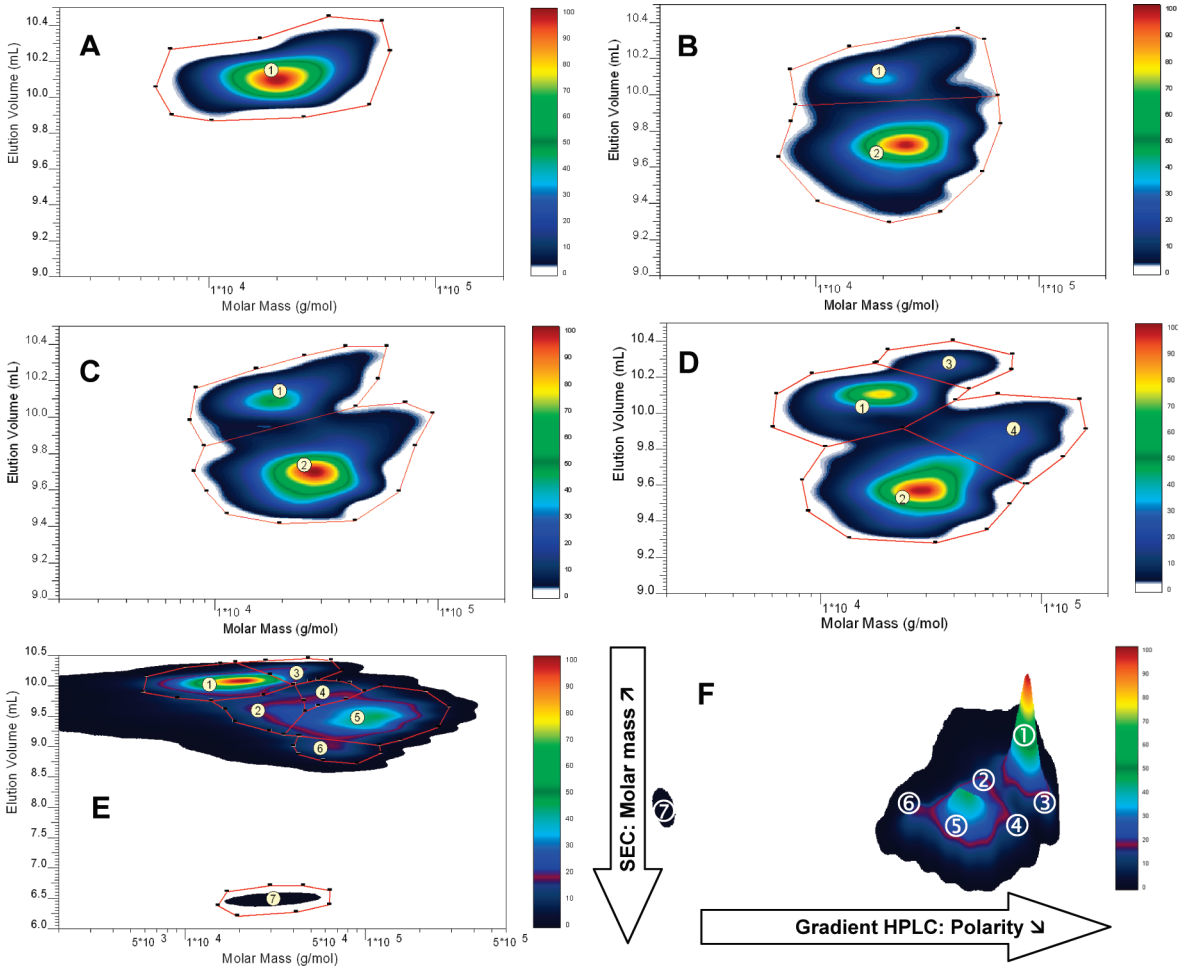
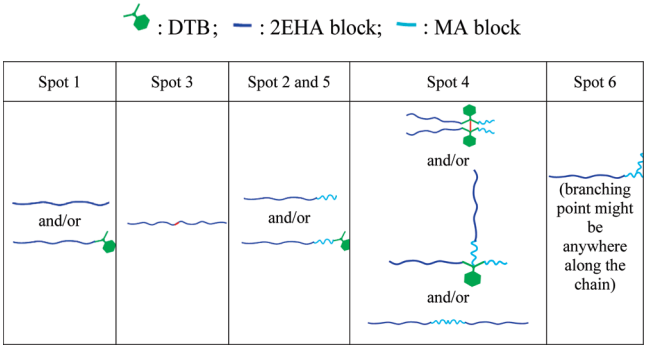


Figure 8. 2D-LC contour diagrams of (A) P2EHA macro(RAFT agent), (B) COPO-1h, (C) COPO-2h, (D) COPO-4h, and (E) COPO-8h. (F) is a rotation of 90° and inclination of 35° of 2D-LC plot from sample COPO-8h. 1st dimension: gradient HPLC with 0 to 70% THF in methanol in 200 min at 0.05 mL/min on PLRP-S 5 μm (*Y*-axis). 2nd dimension: SEC with THF at 1.5 mL/min on PL HTS-C (*X*-axis). Calibration: PMMA. Detection: ELSD.

Table 4. Relative Volumes and Average Relative Molar Masses Obtained after Calibration of the System with PMMA Standards for Each Peak of the 2D-LC Contour Diagrams given in Figure 8

samples	peak number	relative peak volume (%)	$\overline{M}_n$ (g/mol)	$\overline{M}_w$ (g/mol)	PDI
A: macro(RAFT agent)	1	100	19 500	23 000	1.18
B: COPO-1h	1	24	20 500	24 000	1.17
	2	76	23 000	27 000	1.17
C: COPO-2h	1	26	19 500	23 000	1.18
	2	74	26 500	31 000	1.17
D: COPO-4h	1	24	17 000	19 500	1.15
	2	59	28 000	32 500	1.16
	3	4	40 000	42 500	1.06
	4	13	64 500	72 500	1.12
E: COPO-8h	1	31	16 500	18 500	1.12
	2	14	27 500	29 500	1.07
	3	5	38 500	40 500	1.05
	4	5	60 000	63 500	1.06
	5	39	89 500	107 000	1.20
	6	5	63 500	68 000	1.07
	7	1	29 000	32 500	1.12

Table 5. Hypotheses of the Most Probable Chain Architectures for Each 2D-LC Spot Presented in Figure 8



The fact that a specific spot (spot 3) can be isolated only in the COPO-4h sample (Figure 8D) does not necessarily mean that the amount of these chains increased with reaction time. The intensity of the P2EHA peak increased for sample COPO-4h as the intensity of the copolymer peak decreased, in agreement with the gradient HPLC experiments. For Figure 8D and 8E, i.*e*. COPO-4h and COPO-8h, respectively, the volume ratio of spot 3 over spots 1 + 3 corresponds in both cases to ca. 14% of the total nonreinitiated chains. A calibration of the ELSD signal versus the mass of polymer injected in 2D-LC should be made to confirm these results.

The diblock copolymer peak, which elutes in gradient HPLC before the macro(RAFT agent) is quite symmetric after the first hour of polymerization (see spot 2 in Figure 8B) but shows a deformation similar to that observed for macro-(RAFT agent) in the next samples (Figures 8C and 8D). This deformation appears as a tail toward higher molar masses

and higher elution volumes (upper right) of the copolymer spot. It is identified as peak 4 in Figure 8D. Two hypotheses could be formulated to explain the formation of larger macromolecules during the RAFT polymerization: (i) the loss of the MA polymerization control creates long macromolecular chains enriched in MA monomer; (ii) either two active chains or two intermediate macroradicals or an active chain with an intermediate radical (see intermediate radical 1 in Scheme 1) are coupled by termination reactions via combination. The second assumption is the most probable one considering the position of the tail which appears in the direction of higher elution volumes. This indicates that the macromolecules detected in the tail are less polar than the expected binary copolymer, whereas the polymerization of the polar MA monomer increases the polarity of the chain. Thus, a decrease in polarity can only be obtained if 2EHA repeat units are added to the macromolecules. This is expected if a coupling reaction occurs.

A hypothesis can be formulated to explain the formation of macromolecules contained in peak 4. If one considers the initial conditions of polymerization, all species are soluble in isododecane: the macro(RAFT agent), methyl acrylate monomer, and the initiator. When the reaction starts by decomposition of the initiator, a few monomer units of MA are polymerized until the so-formed oligoradical reacts with the C=S bond of a macro(RAFT agent) to form a primary intermediate radical (P2EHA-DTB-PMA). Fragmentation will lead to a P2EHA macroradical able to add MA units until further transfer reaction to a macro(RAFT agent). With chain extension, the copolymers become amphiphilic and tend to segregate to form particles because of the poor solubility of PMA in isododecane. It is thus conceivable that not only the dormant amphiphilic block copolymer chains will segregate, but also the macromolecular intermediate radicals, providing their lifetime is sufficiently long with respect to the diffusion process.[30] This can be explained by their enhanced stability[31] imparted by conjugation of the carbon-centered radical with the aromatic substituent. If this indeed happens, those radical species are situated and confined at the particle surface where coupling reactions can take place to form terminated products as presented in Table 5. This coupling reaction is likely to take place mainly at low conversion level, during the nucleation step. The characteristics of such macromolecules correspond to those found by LC analyses:

- higher elution volume in comparison to the expected copolymer molecules as coupling products contain a higher percentage of 2EHA repeat units
- relative molar mass twice that of the expected copolymers

After particle formation, the polymerization will essentially take place within the particles. This will decrease the probability for the macro(RAFT agent) still present in the continuous phase to react and form block copolymer chains. Consequently, this might be a reason why the whole macro(RAFT agent) is not consumed in the present case of dispersion polymerization whereas it is usually consumed quantitatively in solution or bulk polymerization. This feature was already deduced from kinetic data and was presented in our previous article.[25]

The interpretation of the 2D-LC plot of sample COPO-8h is much more complex since at least five spots of copolymer can be detected. Spots 1 and 3 remain present and are (as previously described) attributed to nonreinitiated P2EHA macromolecules. Spots 2 and 4 already detected in the previous samples were also identified. Spot 4 was expected to be found as the chains, which compose this spot, are terminated and should remain present during the whole synthesis. Spot 2 corresponds more to a tail of the main copolymer spot (spot 5). It most probably corresponds to terminated copolymer chains formed during polymerization as it may occur during any radical polymerization. Spot 5 constitutes the main part of the copolymer. Its position, in comparison to the previous main copolymer spot (spot 2 in COPO-4h, Figure 8D) indicates that the copolymerization continues and addition of MA monomers still occurs: the increase in relative molar mass as well as polarity indicate further addition of MA to the polymer chain. Nevertheless its form is no more symmetrical and it is relatively broad along both axes. This suggests that the polymer chains do not grow at the same rate and that the RAFT process does not allow a complete control over polymerization that occur in the present synthesis. A last kind of copolymer is detected and labeled as spot 6. Its elution in the first dimension gradient HPLC (V_e = 9 mL) indicates that this copolymer contains more methyl acrylate repeat units than all other copolymer fractions. Elution in the second dimension is equivalent to a PMMA standard with a molar mass of 63 500 g/mol, lower than the controlled diblock copolymer. The relative volume (5%, see Table 4) and the chemical composition determined by HPLC suggest that this copolymer was not produced via the RAFT process. Some kind of side-reaction was responsible for its formation. According to these observations, a hypothesis for the structure of these chains can be formulated. The higher polarity (i.e., higher relative content of MA) combined with the smaller relative molar mass than spot 5 suggests that these macromolecules could be stars or branched structures containing several MA arms. Such architecture is likely to be due to intra- and intermolecular chain transfer to polymer by backbone H-abstraction followed by reinitiation by the so-formed midchain radicals[32] especially if the polymerization mostly takes place in the dispersed phase.

The average relative molar mass values in Table 4 are in agreement with those calculated from SEC experiments. It should be reminded that relative molar mass values determined in 2D-LC conditions are less reliable than those obtained from direct SEC as column length is divided by a factor of 6 and flow rate is increased from 1 to 1.5 mL/min. The relative molar masses of residual macro(RAFT agent), spot 1, remained constant during the polymerization process, whereas the relative molar masses for the diblock copolymer (spot 2 for samples COPO-1h to COPO-4h and spot 5 for sample COPO-8h) increased with time, indicating the PMA block growth. The determination of the relative molar masses for spot 3 and 4 in Figure 8D was difficult since they appeared as shoulders of the main spots and not as separate peaks. However, the values are approximately twice as high as those of the main spots. This remark concerning the difficulty to integrate spots could also be applied to spots 2, 3, 4, and 6 for Figure 8E.

Table 5 summarizes all architectural hypotheses inferred from the analyses of the four samples by 2D-LC chromatography. The schemes represent the most probable hypothesis for each 2D-LC spot. It is very likely that they are composed of more than only one kind of chain.

2D-LC SEC × LC−CC: Determination of Relative Molar Mass for Each Species. The LC−CC system exhibits a rather low efficiency in terms of size exclusion separation. In order to overcome this problem, a 2D-LC system was set up where SEC and LC−CC separations were coupled. Such system should first separate the macromolecules according to their global size in solution (SEC) and then fractions containing molecules with identical hydrodynamic volumes should be

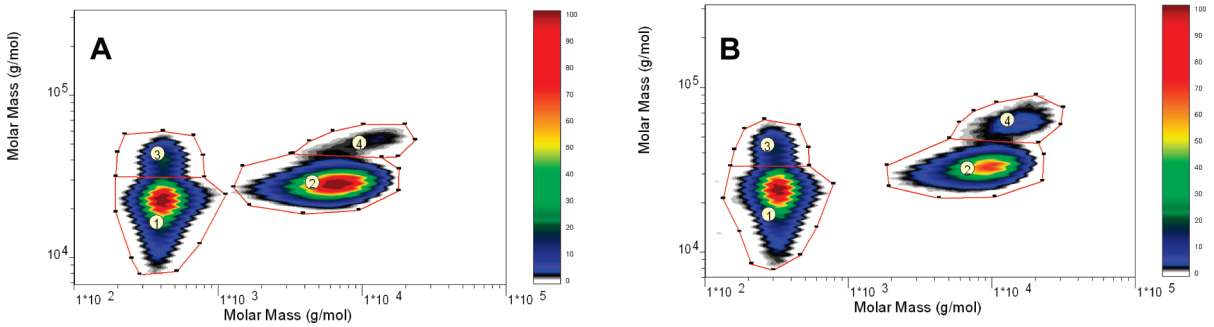


Figure 9. 2D-LC contour diagrams of (A) COPO-2h, (B) COPO-4h. 1st dimension: SEC in THF at 0.04 mL/min on PSS SDV 10^3, 10^4, 10^5 Å. (*Y*-axis). 2nd dimension: LC−CC isocratic ACN:THF 46 54% v/v at 1.0 mL/min on 2 × PLRP-S (*X*-axis). Calibration: PMMA. Detection: ELSD.

Table 6. Relative Volume and Average Relative Molar Mass Obtained after Calibration of the System with PMMA Standards for Each Peak of the 2D-LC Contour Diagrams Given in Figure 9

			PMA block only			total polymer		
sample	peak number	relative peak volume (%)	$\overline{M_n}$ (g/mol) LC−CC	$\overline{M_w}$ (g/mol) LC−CC	PDI LC−CC	$\overline{M_n}$ (g/mol) SEC	$\overline{M_w}$ (g/mol) SEC	PDI SEC
COPO-2h	1	41				20 000	21 500	1.08
	2	51	6100	7200	1.18	28 000	28 500	1.02
	3	6				39 500	40 000	1.01
	4	2	10100	11900	1.18	52 000	52 500	1.01
COPO-4h	1	50				21 000	22 500	1.07
	2	38	8300	9700	1.17	32 500	33 000	1.02
	3	7				42 000	43 000	1.02
	4	5	13700	15800	1.15	61 000	62 500	1.02

separated according to the length of their MA blocks by LC−CC. The advantage of placing SEC in the first dimension is that its resolution is better than that of LC−CC giving more homogeneous fractions.

With the previous analyses, four kinds of macromolecules were detected that were present in the polymer samples taken during the first 4 h: nonreinitiated macro(RAFT agent), dead P2EHA chains coming from termination by combination, the expected binary copolymer, and terminated binary copolymer. We developed a 2D-LC system coupling SEC and LC−CC for P2EHA in order to characterize all four species with regard to molar mass distribution and chemical composition and with regard to the average relative molar masses of the PMA blocks for the copolymers. In this system, SEC separation was used as the first dimension. Each kind of chain elutes according to its hydrodynamic volume in THF. The separation was expected to be similar to that presented in Figure 2. The total mobile phase volume eluting from the SEC columns was transferred into the LC−CC system in fractions of 100 μL each. This second step was meant to discriminate P2EHA homopolymer from copolymer of identical hydrodynamic volume. Using this method we were able to isolate all four polymer species present in the copolymer samples. Figure 9 shows 2D-LC contour diagrams obtained with these conditions for samples COPO-2h and COPO-4h. Table 6 gives the quantification results obtained from these experiments.

Parts A and B of Figure 9 illustrate the expected separation of the four polymer species. Spot assignment remains similar to the previous experiments:

- Spot 1 corresponds to nonreinitiated macro(RAFT agent). Average relative molar masses given in Table 6 fit well with previous SEC results already discussed.
- Spot 3 is attributed to terminated P2EHA by combination. This hypothesis is confirmed by the estimated relative molar mass for this spot which is almost twice as high as that of spot 1. These two spots elute at the system hold-up volume of the second dimension.
- Spot 2 is expected to be the binary copolymer of 2EHA and MA. An increase of the relative molar masses can be noticed from sample COPO-2h to COPO-4h. This indicates the growth of the PMA block. For this spot we also determined the average relative molar masses for the PMA blocks through LC−CC experiments. The relative molar mass values, given in Table 6, roughly correspond to the difference found between nonreinitiated macro(RAFT agent) and binary copolymer relative molar masses.
- Spot 4 is attributed to terminated copolymer species formed by combination of two copolymer intermediate radicals (P2EHA-DTB-PMA) or by combination of an intermediate radical with a growing diblock copolymer, or by combination of two growing diblock copolymers. Average relative molar masses obtained from SEC (first dimension) again confirm the idea of chain termination by combination as relative molar masses are doubled from spot 2 to spot 4. For both samples, the polydispersity for spot 4 remains very low as well. This tends to indicate that macromolecules forming this peak resulted from controlled polymerization. Uncontrolled growth of the PMA block should have led to copolymers with higher polydispersity. The PMA block average relative molar masses determined for this spot are lower than expected. This might be caused by the architecture of the macromolecules (see Table 5 "spot 4"). Therefore, it is likely that, even if these P2EHA blocks should have been chromatographically invisible, they played a role and slightly increased retention time, i.e. decreased relative molar masses of the PMA blocks. LC−CC would give a good estimation of the MA block length in the case of a diblock copolymer. In the case of more complex architectures, where the MA repeating units are not located in one single block, the accuracy of the molar mass determination decreases. It is most probably the reason why the relative molar masses found for the MA block are smaller than expected.

Table 7. Estimation of the Amount of Polymer Contained in the Size Exclusion Chromatograms of Samples COPO-2h and COPO-4h (Figure 2) Using the Results of LC−CC and SEC × LC−CC

	polymer type	amount in total polymer from LC−CC (mass %)	percentage determined from SEC × LC−CC (from Table 6) (%)	estimated amount of polymer contained in SEC shoulder (Figure 2) (mass %)
COPO-2h	P2EHA	35.9 (Table 2)	$\frac{\text{Spot3}}{\text{Spot1}+\text{Spot3}} = 12$	4.5
	copolymers	64.1	$\frac{\text{Spot4}}{\text{Spot2}+\text{Spot4}} = 4$	2.5
COPO-4h	P2EHA	34.7 (Table 2)	$\frac{\text{Spot3}}{\text{Spot1}+\text{Spot3}} = 12$	4.2
	copolymers	65.3	$\frac{\text{Spot4}}{\text{Spot2}+\text{Spot4}} = 12$	7.8

Thus, the peak assignment corresponds to that done in Figure 8. The schematic representations of the most probable polymer architecture for each spot can be found in Table 5.

It should be reminded that the relative peak volumes given here are determined from ELSD detection which depends on elution volume, chemical composition of the polymers and composition of the mobile phase. Only a comparison of the peak volumes for chemically identical species (spot 1 with 3 and spot 2 with 4) seems to be pertinent. Doing so reveals that approximately the same amount of terminated macro(RAFT agent) chains was present in both samples, relative to the total amount of nonreinitiated P2EHA (Table 6, ratio of spot 3 volume fraction over the sum of spots 1 and 3 volume fractions). This amount was estimated to be 12% of P2EHA homopolymer. On the contrary, the comparison of parts A and B of Figure 9 shows an increase in terminated copolymer chains during polymerization. The amount of terminated copolymer (Table 6, ratio of spot 4 volume fraction over the sum of spots 2 and 4 volume fractions) to the total amount of copolymer was estimated to be 4% for sample COPO-2h and 12% for COPO-4h.

Combining the results obtained with LC−CC after calibration of the ELSD and with 2D-LC, the shoulder found in the SEC experiments (Figure 2) for COPO-2h sample could be estimated to be 7% of the total sample: around 4.5% of terminated macro(RAFT agent) and only 2.5% of terminated copolymers. For sample COPO-4h the shoulder should be composed of approximately 4.2% terminated P2EHA homopolymer and 7.8% terminated copolymers which make roughly 12% of total sample and explain the enlargement of the shoulder. These two steps were complementary to properly estimate the amount of terminated chains in both samples (see Table 7 for the calculations).

SEC−^{1}H NMR: Determination of Chemical Composition. Finally, to confirm the information on macromolecules constituting the shoulder in the SEC chromatograms, we performed SEC analyses coupled online with a Bruker 400 MHz NMR spectrometer.

By monitoring the intensity profiles of the O−CH signals at 3.90 ppm (characteristic for 2EHA) and the O−CH_3 signals at 3.64 ppm (characteristic for MA) as a function of elution volume, we were able to determine the chemical composition of the eluting peaks during the SEC separation. The SEC separation was performed in chloroform instead of THF to avoid any overlap of sample and solvent NMR signals. Even though the column set was not as efficient as the previous one and the new eluent impeded separation efficiency (decrease in solvent quality), a shoulder could nevertheless be seen. Figure 10 presents the SEC chromatogram of sample COPO-4h. The chemical composition is plotted as a function of relative molar mass.

According to Figure 10, small molecules contain a higher amount of 2EHA as compared to large molecules. This likely corresponds to the nonreinitiated macro(RAFT agent) which overlaps with the last eluting part of the copolymer.

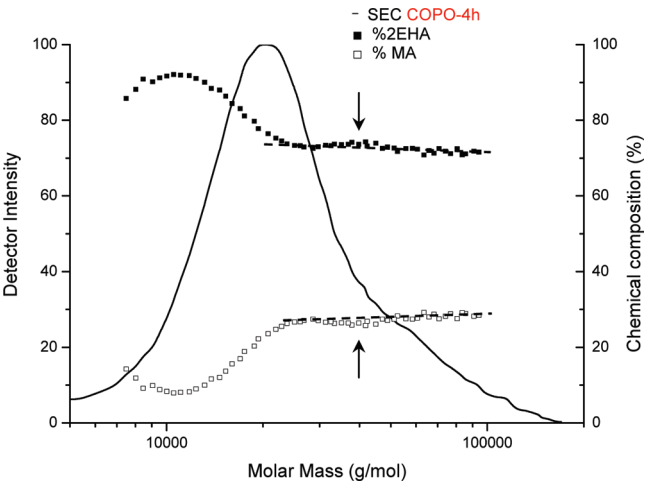


Figure 10. Chromatogram of sample COPO-4h presented with calculated chemical composition from NMR measurements. Columns: PSS SDV 10^4, 10^5 Å, and PL gel mixed-D. Mobile phase: $CHCl_3$. Detection: Bruker NMR 400 MHz. Calibration: PMMA.

As can be seen in Figure 10, for molecules larger than 25 000 g/mol average chemical composition remains nearly constant, i.e. approximately 70 mol % of 2EHA and 30 mol % of MA. This tends to indicate that molecules constituting the shoulder in SEC traces are terminated chains formed by a coupling reaction. Only in this case, the ratio of 2EHA and MA monomers in the large macromolecules could be respected. Indeed an uncontrolled growth of the PMA block would have inversed the chemical composition in favor of MA and no 2EHA monomers are present at this stage of the reaction to keep the ratio constant.

We expected a certain increase in the 2EHA percentage at a relative molar mass of around 40 000 g/mol, corresponding to coupled macro(RAFT agent) radicals. In Figure 10, a small increase in the 2EHA content and a small decrease in the MA percentage can indeed be observed in this region which is stressed by the two arrows. This again proves the formation of macro(RAFT agent) dimers during the polymerization.

Conclusions

In the present work, an analytical strategy has been set up to comprehensively characterize samples taken in the course of the dispersion polymerization of MA using a P2EHA macro(RAFT agent) in order to better understand the deviations that occurred from the controlled polymerization system in solution.

SEC and gradient HPLC experiments already revealed certain heterogeneities of the products in terms of molar mass and chemical composition, respectively. Coupling these two techniques in a 2D-LC system gave more precise information on these byproduct. Nonreactivated macro(RAFT agent)s as well as terminated copolymers were detected. The behavior of the latter molecules in

the 2D-LC experiments provided more precise information on their chemical composition.

Using LC−CC separation it was possible, after calibration of ELSD, to quantify the amount of nonreactivated macro(RAFT agent). Combining these results with chemical composition quantifications obtained from ^{1}H NMR allowed the percentage of 2EHA present in the copolymers to be calculated.

A second 2D-LC method coupling SEC with LC−CC separation was developed. After molar mass calibrations of both dimensions it was possible to characterize precisely all four components identified in the samples, especially the MA block length in the copolymers.

Finally online coupling of SEC with ^{1}H NMR allowed us to determine the average chemical composition as a function of relative molar mass. This was important to confirm the previous results.

With this information and knowing the conditions of the synthesis it was assumed that the dispersed state restrain the RAFT control over the polymerization. The main reason for this loss of control seems to be due to an irreversible termination between intermediate radicals at the interface of the dispersed particles.

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