ORIGINAL PAPER

Quantification of identical and unique segments in ethylene-propylene copolymers using two dimensional liquid chromatography with infra-red detection

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ABSTRACT

Hyphenating high temperature high performance liquid chromatography (HT-HPLC) with high temperature size exclusion chromatography (HT-SEC) (high temperature two dimensional liquid chromatography (HT-HPLC x HT-SEC or HT 2D-LC)) leads to an isocratic elution in the second dimension, which in turn enables to use IR detector (quantitative detection) for monitoring the eluting polymers. Experimental data obtained from HT 2D-LC with IR detector are usually presented as contour plots, which can be mathematically described in matrices. Quantitative data about chemical composition, molar mass and concentration of all the segments, which are present in a polymer, can be obtained, after calibrating the HPLC separation (HPLC elution volume vs chemical composition), SEC separation (SEC separation vs molar mass) and response of the IR detector (IR response vs mass of the polymer). A new procedure based on subtraction and addition of matrices is described, which enables quantitative comparison of different polymer materials. This procedure enables to determine, which components are present in both materials (i.e., identical components or segments) and which are present only in one from both the materials (i.e., unique segments). Moreover, molar mass distribution, as well as chemical composition distribution of both identical and unique segments is evaluated from experimental data. The procedure was applied on two different ethylene-propylene copolymer samples. **Polyolefins J (2016) 3: 119-133**

Keywords: ethylene-propylene copolymer; high temperature two-dimensional liquid chromatography; quantitative detection; infra-red detection; size exclusion chromatography

INTRODUCTION

High performance liquid chromatography (HPLC) using solvent gradients has been used for decades to study the distribution of molecular heterogeneities (e.g., chemical composition distribution (CCD)) in polymers [1]. In HPLC, an interactive stationary phase is used for the selective adsorption of the polymer from a specific mobile phase, followed by desorption of the adsorbed polymer at a particular solvent composition

and temperature [2-4]. Size exclusion chromatography (SEC) is routinely used to determine the molar mass distribution (MMD) of polymers [5, 6]. The separation is entropy driven, based on the size dependent exclusion of macromolecules from the pores of a stationary phase [5, 6].

The relationship between the MMD and other molecular heterogeneities of polymers (e.g., CCD x MMD) can be studied by hyphenating HPLC and SEC. This concept, known as two-dimensional liquid

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chromatography (2D-LC), was introduced to the characterization of polymers by Balke [7, 8] and has been elaborated by others [9-12]. Room temperature two dimensional liquid chromatography (RT 2D-LC) has become a routine technique for many polymers, which are soluble at room temperature. The temperature range of HPLC-separations for polymers has recently been extended to temperatures as high as 160 °C, which enables to analyze semi-crystalline polyolefins. Corresponding multidimensional separations (HPLC x SEC) have since then been reported as well [13-21]. This advance became possible with the discovery that polyolefins can be reversibly adsorbed on porous graphite as stationary phase in dependence on their composition and microstructure, and subsequently be desorbed by a solvent gradient [2, 4].

Qualitative and quantitative information about the molecular heterogeneities of polymers can be obtained by coupling various types of detectors to the chromatographic separation. For the case of HPLC, evaporative light scattering (ELS) detection is widely used for monitoring the effluent as it offers the advantage that the solvents of a mixed mobile phase are evaporated and thus not detected, while polymers are [3, 16, 20-29]. The response of the ELS detector, however, may depend on several parameters, including the nature and composition of the mobile phase and the polymer and it is non-linear with respect to the concentration of the polymer [30]. These shortcomings severely limit the use of an ELS detector for quantitative analysis. NMR has been used in online mode to HPLC, SEC and 2D-LC for quantitative analysis of polymers at room temperature [31-35] and high temperature [36, 37].

For SEC, which uses an isocratic mobile phase, refractive index (RI) and UV detectors are widely used for quantitative analysis for a wide range of polymers soluble at room temperature. In the case of high temperature SEC (HT-SEC) the use of aromatic solvents as mobile phase prevents the use of UV detection. Instead, filter based infra-red (IR) detection has been extensively used for quantitative analysis with HT-SEC [6], as well as for crystallization based techniques like crystallization analysis fractionation (CRYSTAF) [38], temperature rising elution fractionation (TREF) [39] and crystallization elution fractionation (CEF) [40] as its response varies linearly with the concentration of an analyte.

Generally, the detection principles of SEC can

be applied to the corresponding two dimensional separations. Yet, it has to be kept in mind that the solvent used in the first dimensional separation usually contains aliphatic protons i.e., it is detected by the filter based IR-detector. The chromatographic conditions in HT 2D-LC have thus to be optimized in a way that the solvent peak does not interfere with the peak corresponding to the eluting fractions of a polymer analyte.

In most of the cases, the results from 2D-LC have been presented as contour plots and compared qualitatively. The relative volume of spots in different contour plots was compared to derive quantitative information about constituents of samples, yet this procedure does not provide quantitative information about segments, which are present in both samples (i.e., species having either identical molar mass and chemical composition), and segments, which are present only in one sample out of compared samples (different or unique segments). In the case of 2D-LC-NMR also the information about the molecular heterogeneities in different polymers was obtained in the form of contour plots [35].

In this work EP copolymers with different average chemical compositions will be analyzed using HT 2D-LC/IR and a method for quantifying the identical and unique segments in the samples will be described. The contour plots corresponding to these EP copolymers will be created and the matrices corresponding to those contour plots will be used for the quantification.

EXPERIMENTAL

Polymer samples

Linear polyethylene (PE) standards were obtained from Polymer Standards Service (Mainz, Germany). The EP copolymer samples were obtained from SABIC (Geleen, the Netherlands) [41]. The characteristics of the polymers samples are summarized in Table 1. The samples were dissolved at 160 °C for 1-2 hours in the relevant mobile phase.

Mobile phase and stationary phase

TCB and 1-decanol were obtained from Merck, Darmstadt, Germany. TCB was distilled prior to use, 1-decanol was used as received. A HypercarbTM column (4.6 mm \times 250 mm I.D. \times L.) containing porous graphitic carbon with an average particle

Polymer	M _w [kg/mol]	M _p [kg/mol]	C ₂ content [wt. %]	Ð
PE ¹⁸¹	181	126	100	1.59
PE ¹¹⁶	116	75	100	1.4
PE ^{84.5}	84.5	73	100	1.28
	60	55	100	1.47
PE ^{36.5}	36.50	33.5	100	1.31
PE ^{16.5}	16.5	22	100	1.37
PE ^{2.2}	2.2	2.0	100	1.1
EP _{59.7}	125	114	59.7	2.5
EP 39.8	125	117	39.8	2.7
EP _{10.4}	165	147	10.4	2.1

Table 1. Polymer samples used for the experiments.

diameter of 5 μ m, a surface area of 122 m²/g and 250 Å pore diameter from ThermoFisher Scientific (Dreieich, Germany) was used in the first dimension for the separation according to CCD. A PL Rapide H column (7.5 mm × 150 mm I.D. × L.) from Agilent (Waldbronn, Germany) was used in the second dimension for the separation according to MMD in HT 2D-LC.

High temperature two-dimensional liquid chromatography

A high-temperature chromatographic instrument from PolymerChar (Valencia, Spain) was used for all experiments. This instrument was modified: Two solvent selector valves, pump selector and one column selector valve were added, which enabled to select a solvent as well as a column of interest. Moreover, a method selector valve was mounted into the oven (Figure 1), which enabled to realize HT-HPLC, HT-SEC and HT 2D-LC measurements in the same instrument.

The chromatograph was equipped with an autosampler for heating and injecting the samples

into the first dimension column. Two separate ovens were used for heating the HPLC and SEC columns, capillaries and valves. Two pumps, one binary and another one isocratic, equipped with vacuum degassers (Agilent, Waldbronn, Germany) were used for pumping the solvents in the columns as shown in Figure 1. An electronically controlled 8 port valve (VICI Valco instruments, Houston, Texas, USA) was used for transferring fractions from the first column (HPLC) to the second column (SEC). The injection loop (HPLC) and the transfer loop (SEC) had a volume of 250 µL and 100 µL, respectively. An IR4 detector (PolymerChar, Valencia, Spain) and an ELS detector (PL-ELS 1000 from Polymer Laboratories, Church Stretton, England) were used for monitoring the effluent (Figure 1). The dual wavelength IR4 detector has two filters (IR1 and IR2), which are tuned to specific wavelengths: IR1 monitors the overall concentration of a polymer (i.e., -CH- groups), while IR2 is tuned to measure the presence of methyl (-CH₂) groups in a polymer [12]. The ELS detector nebulized the mobile phase at 160 °C in stream of nitrogen with flow rate 1.5 L/min and subsequently the mobile phase was evaporated at temperature 260 °C. The parameters used for the HT 2D-LC measurements are summarized in Table 2.

The parameters summarized in Table 2 were calculated according to the relationships shown in Equations 1-3:

$$V_{\rm TL} = F_{\rm HPLC} * \Delta t \tag{1}$$

$$N_{\rm HPLC} = V_{\rm grad} / V_{\rm TL}$$
 (2)



Figure 1. Scheme of the instrument used for the measurements. Method selector: Position for 2D-LC (a) and HPLC (b) are shown in the figure. (color version is available in online version)



2D-LC method	HPLC flow rate [mL/min]	SEC flow rate [mL/min]	Interval between two injections into SEC column [min]	Transfer loop volume [µL]	Number of fractions (10 mL gradient volume)	Time for 2D-LC analysis [min]
No. 1	0.10	2.5	1	100	100	100
No. 2	0.05	2.5	2	100	100	200
No. 3	0.02	2.5	5	100	100	500
No. 4	0.02	1.5	5	100	100	500

Table 2. Parameters of HT 2D-LC measurements.

$$t_{\rm HT 2D-LC} = N_{\rm HPLC} * \Delta t \tag{3}$$

where V_{TL} is the transfer loop volume, Δt is interval between two SEC injections, F_{HPLC} is the solvent flow rate in HPLC column, N_{HPLC} is number of HPLC fractions, V_{grad} is volume of the gradient and $t_{HT 2D-LC}$ is time required for one HPLC analysis.

The volume of the gradient 1-decanol \rightarrow TCB was kept constant (10 mL), which ensures constant selectivity of the HPLC separation. Data were collected with WinGPC unity software (PSS, Mainz, Germany) and the contour plots and matrices were generated with Origin 9.1 software. Standard deviation (S) in different data sets was calculated using Equation 4 by injecting the samples three times.

$$S = [(\sum (Y_i - \mu)^2)/N]^{1/2}$$
(4)

where Y_i is the value of ith data in full data set (i = [1,2, ...,N]), μ is the average of the data set and N is the total number of points in the data set.

High temperature SEC

The PL Rapide H column was connected with the column selector valve and TCB was selected as the mobile phase. An injection loop of 100 μ L was used. The solvent flow rate in the SEC column was 2.5 mL/min, one complete SEC analysis required 1-5 minutes. No stabilizer was used.

High temperature HPLC

The program of the gradient elution used for the

Desorption promoting	Linear gradient [min]			
solvent [vol. %]	Pump	ELS detector		
0	0	0		
0	3	6.7		
100	13	16.7		
100	17	20.7		
0	20	23.7		
0	45	48.7		

 Table 3. Parameters of HT HPLC measurements.

HPLC analysis is shown in Table 3. A linear gradient 1-decanol \rightarrow TCB was used with a flow rate of 1 mL/ min. 1-decanol was pumped after 17 min (Table 3) with the aim to remove TCB from the column and to prepare the column for the next analysis. The total delay volume of the HT HPLC system (3.7 mL) was determined according to Ginzburg et al. [17]. This value enables to calculate composition of the binary mobile phase in the detector (Table 3).

A stabilizer was not added into the mobile phase or into the sample solution. Degradation of polymer samples is not expected, as the polymer will be adsorbed in the column most of time and finally it will be eluting at a low flow rate [42].

RESULTS AND DISCUSSION

Comparison of ELS detector and IR-detector responses from HT 2D-LC analysis

PE116 was dissolved in 1-decanol and injected into the HypercarbTM column using the solvent gradient program shown in Table 3. A comparison between the response of the ELS detector and that of the IR4detector is shown in Figure 2.

The numbers of peaks corresponding to PE obtained with both detectors are the same, yet it can be observed that the IR response for the polymer is significantly lower compared to that for the solvent (1-decanol).

Optimization of the solvent flow rate in the first dimension of HT 2D-LC

While the solvent is "invisible" due to evaporation when using an ELS detector, interference of the solvent peak with the polymer peak may become an obstacle when using IR-detection in HT 2D-LC. This solvent peak could be eliminated from the chromatograms if an IR transparent solvent for the tuned wavelength region, which at the same time supports the adsorption of the analyte, would be found. The other option is to improve the separation between the peak of the solvent



SEC elution volume [mL] **Figure 2**. Comparison of the response of PE116 obtained with the IR4-detector (IR1 signal) and ELS detector. HT 2D-LC method No. 4 was used (Table 2). The broken line indicates injection of a HPLC fraction into the SEC column.

and that of the polymer. Therefore, the HPLC flow rate has to be optimized with the aim to separate the solvent and the polymer peak from each other in the SEC column. Roy et al. [18] have also demonstrated a positive effect on the separation of both peaks, when a SEC column with larger inner diameter was used (10 mm instead 4.6 mm). SEC-traces obtained from HT 2D-LC analysis of PE116, using different chromatographic parameters, are shown in Figure 3.

The peaks of PE were overlapping with the peak of 1-decanol from the previous injection at an HPLC flow rate of 0.10 mL/min (Figure 3a). On the contrary, the peaks corresponding to 1-decanol and the polymer were well separated from each other when the HPLC flow rate was reduced to 0.05 mL/min (Figure 3b), as this increases the time interval between two injections into the SEC column. Lowering the flow rate in the HPLC column further to 0.02 mL/min (Figure 3c) leaded to better separation of PE fractions from the solvent peak. The size of the separation window in SEC is shown in Figure 4 for different combinations of flow rates in HPLC and SEC.

The size of the separation window at different solvent flow rate combinations (HPLC x SEC) is



Figure 3. SEC-traces of PE116 recorded with IR4 detector (IR1 signal) at the following flow rate in HPLC column: (a) 0.10 mL/min (2D-LC Method 1 described in Table 2 was used), (b) 0.05 mL/min (Method 2) and (c) 0.02 mL/min (Method 3). The dotted lines in the figures indicate the injection signals.

presented in Table 4.

Reducing the HPLC flow rate increased the size of the separation window (Figure 4), while reducing the SEC flow rate decreased the size of the separation window (Figure 4d). The largest separation window is observed at an HPLC flow rate of 0.02 mL/min (Figure 4c). The HPLC flow rate of 0.05 mL/min is chosen as optimum flow rate as it provides sufficient separation window for EP copolymers to elute and also reduces the analysis time (Figure 4b). Also the binary pump is more reliable at 0.05 mL/min compared to 0.02 mL/min.

Representation of the quantitative data as a contour plot

EP59.7 was analyzed with HT 2D-LC and the SECtraces are shown in Figure 5a, b. The solvent peaks were excluded and only the SEC-traces corresponding to the polymer were selected (Figure 5c) to generate a color coded contour plot (Figure 5d).

The sample eluted in 15 fractions (fractions 113 - 127) from the HPLC column i.e., 15 SEC analysis were used for construction of the contour plot (Figure 5d). This contour plot can be represented as a matrix with i number of rows (number of rows equal to



Figure 4. Separation window: (a) 2D-LC method No. 1 (Table 2), (b) method No. 2, (c) method No. 3 and (d) method No. 4. Flow rates HPLC × SEC are stated in the figure. The dotted lines in the figures indicate the injection signals and the blue circles indicate the separation window available for a polymer sample. Note: Pure 1-decanol was injected.

number of points or the IR responses from one SEC analysis) and with j number of columns (number of columns equal to number of the HPLC fractions) i.e., with $i \times j$ number of elements ($E_{i,j}$). The mass of polymer, the average molar mass and the average ethylene content corresponding to each element $E_{i,j}$ as well as to each HPLC fraction can be calculated by applying corresponding calibrations (concentration of polymer vs. response of the IR detector, average molar mass vs. SEC elution volume and average chemical composition vs. HPLC elution volume).

Table 4. Separation window at different flow rates in HPLCand SEC.

HPLC flow rate [mL/min]	SEC flow rate [mL/min]	Separation window [mL]		
0.1	2.5	0.5		
0.05	2.5	2.27		
0.02	2.5	3.93		
0.02	1.5	3.27		

Calibrations

Ortin et al. demonstrated that the response of the IR4 detector (IR₁ signal) is independent of the CC of EP copolymers [12]. The mass of polymer in each element of the matrix can be calculated from a calibration curve, which expresses the dependence between the mass of polymer and the response of the IR detector. Such a calibration curve can be obtained by injecting solutions with different concentrations of the polymer and plotting the IR response with respect to the injected concentration. An alternative way is to inject a polymer solution with known concentration and partition the SEC elugram into identical slices (e.g., 100 μ L). This method can be applied for both symmetrical and asymmetrical peaks because the length of the slices is small. The average IR response of the individual slice can then be plotted against the mass of polymer eluting in that slice, which can be derived from the total injected mass of polymer.



Figure 5. SEC-traces for sample EP59.7: (a) Response of the IR4 (IR1 signal), (b) zoomed at the central peaks, (c) stacked SEC-traces (fraction 113 – 127), (d) color coded contour plot. Concentration: 1 mg/mL, HPLC flow rate: 2.5 mL/min, SEC flow rate: 0.05 mL/min. 2D-LC method No. 2 was used. (color version is available in online version)

An advantage of this approach is that only a single injection is required. A solution of sample EP59.7 in TCB was injected into the SEC column and the elugram of the sample is shown in Figure 6.

The total area of the polymer peak (Figure 6a) corresponds to the mass of the polymer injected into the SEC column and eluted over a volume of 1400 μ L. This elugram was divided into 100 μ L slices (the volume of the injection loop), and the area corresponding to each volume fraction was calculated and compared with the total area of the elugram to obtain the mass of polymer eluting in each fraction (Figure 6b). Using this method a relation between the IR response and the mass of polymer eluted was obtained (Figure 7).

A linear relationship between the IR response and the mass of the EP copolymer (Figure 7) is expressed in Equation 5. The reproducibility of the results was checked by injecting the sample three times and the standard deviation observed in the IR response is shown in Figure 7.

$$H_{ij} = 0.3237 * m_{ij}$$
(5)

$$m_{i,j} = H_{i,j} / (0.3237) \tag{6}$$

Equation 6 enables to calculate mi,j, i.e., the mass of the polymer in an element Ei,j of the matrix (i.e., in volume of one HPLC fraction = 100 μ L), when an IR response (H_{i,j}) for that element is known. The smallest concentration of polymer, which can be reliably detected i.e., the limit of quantification (LOQ) [43], was calculated by determining the signal to noise ratio at lower concentrations. A signal to noise ratio of ~ 10 corresponds to the LOQ [43]. The LOQ for the IR4 detector was calculated as 0.003 mg/100 μ L.

The relation between the SEC elution volume and the molar mass at peak maximum (M_n) was obtained





Figure 6. SEC chromatogram for sample EP59.7: (a) Area of the peak, (b) Area corresponding to 100μ L in the 6th fraction of the chromatogram. Solvent flow rate in SEC: 2.5 mL/min, injection loop: 100μ L.

by analyzing PE standards (dissolved in TCB) with the SEC column (Figure 8).

The calibration of the SEC separation is presented in Figure 8 and is expressed with equation 7 (R2 = 0.993):

$$M_{p} = 14.33 \times V_{SEC} - 2.4 \times (V_{SEC})_{2} - 15.95$$
(7)

With the aim to calibrate the HPLC separation, three copolymer samples and one PE standard (Table 1) were analyzed with HT 2D-LC at optimized chromatographic conditions (Method No. 2 in Table 2). The relation between the HPLC elution volume at peak maximum (E_{Pmax}) with respect to the average chemical composition of the EP copolymers is illustrated in Figure 9.

A linear relationship between the HPLC elution volume and the ethylene content was obtained (Figure

9) which could be expressed as Equation 8. The reproducibility of the results was checked by injecting each sample three times and the standard deviation in E_{Pmax} for each sample is shown in Figure 9.

$$E_{P_{max}} = 9.843 + 0.0416 * EC$$
(8)

$$EC = (E_{P_{max}} - 9.843) / (0.0416)$$
(9)

where EC represents the ethylene content in wt. % in an EP copolymer.

Applying the three calibrations (Figures 7 – 9, equations 6, 7, 9), the mass fraction of polymer, the Mp and the weight average ethylene content (EC_w) related to the HPLC fractions (Figure 6c) of the sample EP59.7 (fraction 113 – 129) were calculated and results are summarized in Table 5.



Figure 7. Relation between the IR response (peak height) and the mass of sample EP59.7 copolymer.



Figure 8. Calibration of the SEC separation with PE standards obtained with HT-SEC/IR4 (IR1 signal): The Mp of PE standards versus elution volume. Concentration: 2 mg/mL. SEC solvent flow rate: 2.5 mL/min.



Ethylene content [wt.%]

Figure 9. Calibration of the HPLC separation obtained with HT 2D-LC/IR4 (IR1 signal): E_{pmax} versus the average chemical composition of the EP copolymers.

The results in Table 5 show that the initial fractions from the HPLC column have a lower M_p compared to the later fractions and most of the polymer sample eluted in the middle fractions.

Matrix corresponding to a contour plot

The data presented in the form of matrices enable to perform mathematical operations with the aim to investigate differences in the molecular heterogeneities

Number of	M Electron el l	EC [ud 9/1	Mass fraction	
fraction	wi _p [kg/moi]		[wt. %]	
113	32.35	35.02	1.0	
114	49.75	37.42	1.7	
115	59.13	39.83	2.1	
116	73.52	42.24	3.1	
117	67.89	44.64	4.1	
118	48.18	47.04	5.9	
119	64.86	49.48	6.8	
120	71.22	51.85	9.6	
121	74.64	54.25	11.8	
122	77.55	56.66	17.2	
123	79.29	59.06	17.6	
124	77.88	61.47	13.3	
125	95.35	63.87	4.2	
126	160.95	66.27	1.5	
127	251.94	68.68	0.1	

Table 5. M_p , EC, and mass of polymer for the HPLC fractions of sample EP59.7.

of polymers, which is not possible with the contour plots. Using the calibrations illustrated in Figures 8 and 9 i.e., equations 7 and 9, the x and y axes were recalculated for the contour plots of EP59.7 and EP39.8. In the same sense the IR response was also recalculated to the mass of polymer using equation 6.

The data used for the creation of these contour plots (Figure 10) are shown in the form of matrices, where the number of rows (x-axis of the contour plot) correspond to the number of points (IR-response) from one SEC analysis and the number of columns (y-axis of the contour plot) correspond to the number of fractions from the HPLC column, in Figure 11.

The matrices shown in Figure 11 contain the quantitative data, and the mass of EP copolymer for a particular chemical composition and/or a particular molar mass can be identified from the matrix.

Application of the matrix approach

The EP copolymers of varying average ethylene content can also contain identical segments i.e.,



Figure 10. 2D-contour plot with recalculated values for (a) sample EP59.7 and (b) EP39.8. Concentration: 2 mg/mL, HPLC flow rate: 2.5 mL/ min, SEC flow rate: 0.05 mL/min. 2D-LC method No. 2 was used. (color version is available in online version)







(b)

Figure 11. Matrix corresponding to the recalculated 2D-contour plots (for Figure 10): (a) sample EP59.7 and (b) EP39.8.

macromolecules with identical molar mass and chemical composition. The amount of the common segments could be more extensive for the broadly distributed samples. Samples EP39.8 and EP59.7 were analyzed using HT 2D-LC/IR. The matrix corresponding to the contour plot for sample EP39.8 (Figure 11b) was subtracted from the matrix corresponding to the contour plot of sample EP59.7 (Figure 11a), to obtain information about the common segments of the two copolymers. The difference matrix and the three dimensional surface plots generated from this (for unique and identical segments in EP39.8 and EP59.7) are shown in Figures 12-14.

The matrix in Figure 12 shows negative values (shortage part) for the part present only in EP39.8, and positive values (surplus part) for the part present only in EP59.7. The copolymers contained identical segments, which are not visible in the matrix in

Figure 12. However, the mass fraction of the identical segments in both the copolymers can be calculated by using the data from the matrices corresponding to their contour plots (Figure 11) and the data from the subtraction matrix (Figure 12).

It is valid that mass of sample EP39.8 can be calculated from the corresponding matrix as sum of identical segments with mass I and unique segments with mass D_1 :

$$A = D_1 + I \tag{9}$$

The similar equation may be written for EP59.7:

$$\mathbf{B} = \mathbf{D}_{2} + \mathbf{I} \tag{10}$$

where mass B may be calculated from the corresponding matrix.

Mass of both samples T may be obtained after summation of both matrices using equation 11,

$$T = A + B = D_1 + D_2 + 2I$$
(11)

while mass C can be calculated from the subtraction of matrices:

$$C = A - B = D_1 + D_2$$
 (12)

Finally, mass of the identical segments (I) in both the samples can be calculated using equation 13:

$$I = (T - C)/2$$
(13)

Using Equation 13 the mass fraction of identical segments in both the copolymers was determined and



Figure 12. Matrix obtained after subtraction operation (EP59.7 – EP39.8). 2D-LC method no. 2 was used. Note: Negative values in the matrix show the unique segments in sample EP39.8 and positive values show the unique segments in EP59.7.



Figure 13. Plots obtained after subtraction (EP59.7 – EP39.8) showing unique segments in both the copolymers: (a) Three dimensional surface plot, (b) Projection of the SEC elution and (c) Projection of the HPLC elution. Note: Negative peaks in the figures correspond to the unique segments in EP39.8 and the positive peaks indicate the unique segments in EP59.7.

the corresponding contour plot is shown in Figure 14. Both the samples contained 10.5 wt. % of identical segments in the chemical composition range 39.8 - 47.0 wt. % (ethylene content).

The number and weight average values of both the molar mass distribution (M_n, M_w) and the chemical composition distribution (EC_n, EC_w) were calculated using Equations 14-17, as described by Kebritchi at al [44]. The $M_{i,j}$ and the $H_{i,j}$ values were obtained from the corresponding matrices.

$$M_{n} = \left[\sum(M)_{i,j} * H_{i,j}\right] / \sum H_{i,j}$$
(14)

$$\mathbf{M}_{w} = \left[\sum(\mathbf{M})_{i,j}^{2} * \mathbf{H}_{i,j}\right] / \left[\sum(\mathbf{M})_{i,j} * \mathbf{H}_{i,j}\right]$$
(15)

$$EC_{n} = \left[\sum(EC)_{i,j} * H_{i,j}\right] / \sum H_{i,j}$$
(16)

$$EC_{w} = \left[\sum (EC)_{i,j}^{2} * H_{i,j}\right] / \left[\sum (EC)_{i,j} * H_{i,j}\right]$$
(17)

The calculated average molar mass (M) and average ethylene content (EC) of the identical and unique segments are summarized in Table 6.

Data in Table 6 reveal that the identical segments in both polymers have lower average molar mass compared to the unique parts. The unique segment



Figure 14. Plots obtained after subtraction (EP59.7 – EP39.8) showing the identical segments in both copolymers: (a) Three dimensional surface plot, (b) Two dimensional contour plot. (color version is available in online version)

in EP59.7 has larger average chemical composition, while the unique segment of EP39.8 has lower average chemical composition in comparison with the identical segments.

The matrix approach enables to identify the identical and the unique segments within two polymers quantitatively. In this way differences in both CCD and MMD of complex polymer samples may be quantitatively visualized, evaluated and compared. Such quantitative data about differences in the molecular heterogeneities are an important step towards establishing structure-property relationships.

CONCLUSION

HT 2D-LC with IR detection was used for the quantitative analysis of ethylene-propylene copolymer samples. The adsorption promoting solvent used in the first dimension of the HT 2D-LC was not IR transparent and gave intensive solvent peaks, which overlapped with the peaks of polymer. This problem was overcome by optimizing the solvent flow rate in the first dimension of the separation. The experimental

data from 2D-LC: elution volume in HPLC, elution volume in SEC and response of the IR detector, were organized into a matrix. Calibrations of the HT-HPLC separation with respect to chemical composition, the HT-SEC separation with respect to molar mass and the response of IR detector with respect to mass of polymer injected were plotted to use them for quantitative analysis. The calibration curve for the IR response with respect to concentration of the polymer was obtained using a new method, which required only one injection. The limit of quantification was identified to check the limit of polymer mass, which can be reliably detected. The standard deviations in all the calibrations were calculated and observed to be very small.

The matrices corresponding to the contour plots can be used to determine the mass of EP copolymer of any particular chemical composition and molar mass of interest. Subtraction of matrices corresponding to different EP copolymer samples enabled to identify the identical and the unique segments present in the EP samples. Summation and subtraction of both matrices were used to calculate the mass fraction of the identical segments. MMD, CCD as well as average

 Table 6. Average molar mass and average chemical composition of the identical and unique segments identified in two polymer samples.

Polymer sample	EP _{59.7}			EP _{39.8}				
Segments	Identical		Unique		Identical		Unique	
Mass fraction [wt. %]	10.5		89.5		10.5		89.5	
Average M [kg/ mol]	M _n	M _w	M _n	M _w	M _n	M _w	M _n	M _w
	79.6	112.6	107.1	141.7	79.6	112.6	107.6	147.5
Average EC [wt. %]	EC _n	EC	EC _n	EC	EC _n	EC	EC _n	EC
	44.32	44.45	59.6	59.92	44.32	44.45	36.08	36.89

molar masses and average chemical compositions corresponding to the identical and unique segments were calculated and presented in numerical and graphical forms. The described procedure holds a promise for practical application of HT 2D-LC, because the differences between several polymer materials with identical types of comonomers and microstructures (one sample from a set of samples of interest is chosen as a reference sample) can be compared qualitatively and quantitatively. Moreover, the procedure may be applied on variety of polyolefin materials. Quantification of the common and unique segments for series of polymer samples can lead to better understanding of their structure-property relationships as well as the selectivity of catalysts and processes.

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