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Full copolymer characterization by SEC–NMR combined with SEC–MALDI

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Abstract

Measurements on a series of random copolymers with units of methylmethacrylate, butylacrylate, styrene and maleic-anhydride are performed. A method is used which consists in fractionating the copolymer by size exclusion chromatography (SEC), collecting 30–40 fractions and then recording both the NMR and MALDI spectra of the fractions. In a successive step, bivariate distribution of chain sizes and composition maps are derived from knowledge of the molar mass, weight and composition of the copolymer fractions. The compositional distribution is obtained by summation of the sections of the bivariate distribution which belong to a narrow compositional range.

A model for SEC fractionation of copolymers is developed. It allows to predict the composition and $D(x)$ of the SEC fraction ($D(x)$ is the ratio between the number-average and the weight-average molar mass, x is the fraction number).

Offline SEC–MALDI measurements are performed, in order to measure experimentally $D(x)$ for the random copolymer. The agreement between theoretical and experimental values for $D(x)$ and for the composition of the SEC fraction (as derived from SEC–NMR measurements) is good. The predictions of the model were also compared with literature data for SEC fractionation of a copolyester with units of butileneadipate and butilenesebacate. The agreement between theoretical and experimental values is more than satisfactory.

Keywords: Bivariate distribution; Compositional distribution; Size exclusion chromatography

1. Introduction

Size exclusion chromatography (SEC) is a widely used technique in polymer and copolymer characterization. The standard configuration of an SEC apparatus consists of three parts [1–6], namely a solvent-delivery system (equipped with an injection port) the columns and a differential refractive index (RI) detector.

This basic configuration can be modified by adding an additional detector and this addition can be done in two ways, namely placing the second detector in series or in parallel with respect to the RI detector [1–6]. As a second detector, one can use a viscometer [2], a concentration detector [3], an UV detector [4] or a light scattering (LS) device [5,6].

NMR can be used as a detector for SEC of polymers. SEC and NMR can be connected on-line, using a commercial probe (work in the field of on-line coupling between liquid chromatography and NMR [7,8] has been reviewed), and this technique has been applied to homopolymers [9–11] and copolymers [12–15].

The on-line SEC–NMR technique faces a difficulty. The signal-to-noise ratio (S/N) of the NMR spectrum is related to the strength of the magnetic field (B) by a nonlinear relationship (S/N scales as B raised to 1.5). Thus, when an NMR with a low-field magnet is used, the signal-to-noise ratio of the NMR spectrum is poor, thus one is forced to use high-field magnets (600 MHz or higher) and the cost of the SEC apparatus experiences a huge increase with respect other SEC assemblies. Moreover, the NMR probe for on-line coupling is itself a quite sophisticated piece of equipment and it contributes to the cost. As a consequence, double-detector SEC assemblies are more affordable and their use is more widespread than on-line SEC–NMR.

Furthermore, although NMR is always able to determine the composition of a copolymer fraction (independent from its molar mass value), it provides reliable molar mass estimates only up to 10,000–20,000 Da. One may try to estimate the molar mass from SEC retention times. Specifically, a mixture of five or more polymer samples with the same repeats unit, possessing a narrow MM distribution and known mass (the so-called SEC primary standards), is prepared. The mixture is injected in the SEC apparatus and the resulting chromatogram is recorded. Measuring the elution volumes and plotting them against the logarithm of the mass, the calibration line is obtained. However,

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calibration standards with narrow distribution, known composition and known molar mass are often not available. For this reason, approximate calibration lines are often used, although surprises may show up (especially in the case of high conversion samples, which are complex mixtures), since the calibration is logarithmic (i.e. log(M) is used) and the law of propagation of errors in indirect measurement predicts that the $\bar{M}_n$, $\bar{M}_w$ estimates performed with the use of an inaccurate calibration line become useless and misleading.

Off-line SEC–NMR does not suffer of the above drawbacks. The signal-to-noise ratio is good when medium-field magnets (200 up to 500 MHz) are employed and thus cost of this SEC apparatus is acceptable i.e. of the same order of magnitude of double-detector SEC assemblies.

In order to cope with the difficulty of the conversion from elution volumes to molar masses, one can use a MALDI mass spectrometer and record the MALDI mass spectra of the SEC fractions of homopolymers and copolymers [15–24], since the MALDI technique possesses extraordinary sensitivity and it is able to measure molar masses up to very high values (10^6 Da). Furthermore, in order to have a complete picture, one can record both the NMR and the MALDI spectrum of each SEC fraction [23,24].

Off-line SEC–NMR differs from on-line SEC–NMR, since the former is certainly more labor-intensive and time-consuming. Nevertheless, this difference can be minimized by reducing the number of fractions. Clearly, the reduction cannot go beyond a certain limit, otherwise it will cause a loss of accuracy in the measurement of copolymer properties. The hypothesis that it is possible to reduce the time for copolymer analysis without the cited loss of accuracy was put forward by Murphy et al. [25] in another context (2D-chromatography) and it would be interesting to check whether the hypothesis is valid also in SEC–NMR and to develop a methodology which allows to determine the optimal conditions, namely those where the cited loss of accuracy is still negligible.

In this paper, off-line SEC–NMR, measurements are performed (along with SEC–MALDI) on random copolymers with units of methylmethacrylate, butylacrylate, styrene and maleic-anhydride reacted at high conversion.

In the first series of measurements, the copolymer is fractionated by SEC, fractions are collected and both the NMR and the MALDI spectra are recorded. The results are employed to derive some copolymer properties, such as the composition distribution histogram, which reports the weight fraction of chains with a given composition.

In the second series of measurements, the fractionation conditions are varied and the sampling rate (which is proportional to the total number of fractions) is reduced by increasing the volume of the fraction. The goal of these experiments is to find the optimal conditions, namely the largest volume of the fraction where the above-mentioned loss of accuracy is small.

In order to interpret the resulting data, a model is developed for copolymers obtained by SEC fractionation which takes into account the fractionation conditions and specifically the number of fractions. The model predicts the composition and the ratio $D(x)$ of the SEC fraction ($D(x)$ is the ratio between the number-average and the weight-average molar mass, x is the fraction number). The predictions of the model are compared with SEC–NMR and SEC–MALDI data for the random copolymer with units of styrene and MMA reacted at high conversion.

In SEC of copolymers, a very subtle effect shows up [1,23,24], namely the 'mass coelution' effect. The calibration establishes a correspondence between masses and elution volumes. In the case of homopolymers the calibration process is straightforward, since the cited correspondence is univocal. For compositionally homogeneous copolymer samples, the correspondence is still univocal, since the macromolecules which are found in the sample are similar to each other. The case of compositionally heterogeneous copolymer samples, the calibration process is more complex, since the cited correspondence is not univocal and chains at different masses are coeluted in the same SEC fraction. The predictions of the model concerning the 'mass coelution' effect are analyzed theoretical estimates are compared with literature data for SEC fractionation of a copolyester with units of butileneadipate and butilenesebacate.

2. Theory

In the experiment which we consider, a certain amount of copolymer sample is injected in the SEC apparatus. The sample is a copolymer in which two repeat units A and B are found along the chain. The macromolecular chains are of the type A_mB_n, the size of the chain being $s = m + n$, the mass of the chain being $(m\mu_A + n\mu_B)$ where μ_A and μ_B are the masses of A and B. The mass is approximately $(s\mu)$ where μ is the average mass of the repeat unit, namely $\mu = (\mu_A + \mu_B)/2$.

The chromatographic columns separate the copolymer chains, the detector records the trace, $f(V)$, where V is the elution volume, various fractions are collected and the spectrum of each fraction is recorded. Fraction 1 contains the copolymer solution eluted between $(V_0 + V_1)$ and $(V_0 + 2V_1)$, fraction 2 contains the copolymer solution eluted between $(V_0 + 2V_1)$ and $(V_0 + 3V_1)$, fraction x contains the copolymer solution eluted between $V_{\mathrm{ini}} = (V_0 + xV_1)$ and $V_{\mathrm{fin}} = (V_0 + (x+1)V_1)$.

For chains of the type A_s, one has $V = (\log(s\mu) - a_0(/b_1$, whereas for chains of the type B_s, one has $V = (\log(s\mu) - b_0(/b_1$, where a_0, b_0, b_1 are calibration constants.

The quantity of interest is $c(x, q)$, the molar fraction of A units for chains of size q in SEC fraction x, which turns out to be

$$c(x, q) = c(x, s - s_{\mathrm{mid}}) \qquad (1)$$

where s_{mid} is the size of chains which possess middle dimensions and it is given by $s_{\text{mid}} = (s_{\text{fin}} - s_{\text{ini}})/2$ (here s_{fin}, s_{ini} denote the average sizes of chains eluted at volumes V_{fin} and V_{ini}) and where the molar fraction of A units for chains of size s in SEC fraction x, $c(x, s)$, (see Ref. [26]) is given by

$$c(x, s) = \left(\int mI(x)\delta_s \, \mathrm{d}m \right) / \left(\int sI(x)\delta_s \, \mathrm{d}m \right) \tag{2}$$

where the integration goes from $m = 0$ to s and δ_s indicates that the integration is extended to chains A_mB_n for which $m + n = s$. The quantity $I(x)$ which appears both in the numerator and the denominator is the molar fraction of chains A_mB_n in SEC fraction x. When the coupling between size and sequence is weak, $I(x)$ splits in two parts, namely the size distribution and the composition distribution (see the quantitative description of polymers by Kuchanov [27] and calculations by Tobita [28] for random St–MMA copolymers). In our case, $I(x)$ is given by

$$I(x) = g_1 \exp(y + J)Q \tag{3}$$

where g_1 is a suitable normalization factor, J (the sequence distribution) is given by $J = (m/s - d(/h$, with $d = d_0 + d_1 s + d_2 s^2$, where d_0, d_1, d_2 are parameters which describe the sample's compositional heterogeneity, where h is the width of the compositional distribution, $y = y_1\,log(s) - s/y_2$ where y_1 and y_2 are given by $y_1 = (2\bar{M}_{\text{n}} - \bar{M}_{\text{w}})/(\bar{M}_{\text{w}} - \bar{M}_{\text{n}})$, $y_2 = \mu/(\bar{M}_{\text{w}} - \bar{M}_{\text{n}})$ and $\bar{M}_{\text{n}}$, $\bar{M}_{\text{w}}$ are the number- and weight-average molar masses of the unfractionated copolymer, Q is a function which describes the fact that SEC fraction x contains chains A_mB_n of a given size and it does not contain chains A_mB_n of other sizes. Clearly, Q is always equal to zero, except in a small range of elution volumes between V_{ini} and V_{fin}. When the separation process is extremely efficient, one has

$$Q = H(V - V_{\text{ini}})H(V_{\text{fin}} - V) \tag{4}$$

where the function H is equal to zero when its argument is negative and it is equal to 1 when its argument is positive.

The quantity $c(x, q)$ in Eq. (1) can be expanded in a MacLaurin series around $z = 0$, yielding

$$c(x, q) = c(x) + z\rho_1\rho_2 \tag{5}$$

where $\rho_1 = a_0 - b_0$, where ρ_2 is a parameter related to $\bar{M}_{\text{n}}$, $\bar{M}_{\text{w}}$, and to the chromatographic conditions, namely b_1, V_0, V_1, and where $c(x)$ is the average molar fraction of A units in the fraction.

It is useful to introduce a quantity which measures the difference between the compositional properties of the unfractionated copolymer and those of the SEC fraction. This can be done as follows

$$\phi = \log|c(x) - c_{\text{AVE}}| \tag{6}$$

where c_{AVE} is the average molar fraction of A units in the unfractionated copolymer. It follows from Eq. (1) that

$$\phi = \log(c_3) - \lambda \tag{7}$$

where c_3 is a parameter (of the order of 0.01–0.03 ml) and $\lambda = \log(V_1)$.

Let us denote with $D(x)$ the ratio between the weight-average and the number-average molar mass in fraction x. Using the definitions, $D(x)$ becomes:

$$D(x) = \sum(\mu)^2 I(x) / \sum I(x) \tag{8}$$

When SEC fraction x is mixed together with SEC fraction y, the value of the molar fraction of A units in the resulting fraction $c(z)$, will depend on weight of copolymer in the two fractions, $w_{\text{copo}}(x)$ and $w_{\text{copo}}(y)$ and the value will be intermediate between $c(x)$ and $c(y)$, namely

$$c(z) = k[\nu(x)c(x) + \nu(y)c(y)] \tag{9}$$

where $\nu(x) = w_{\text{copo}}(x)(\mu s_{\text{mid}})$, $\nu(y) = w_{\text{copo}}(y)(\mu s_{\text{mid}})$ and $k = 1/[\nu(x) + \nu(y)]$.

The bivariate distribution reports the weight, $W(s, c_{\text{A}})$, of chains which possess a given size (s) and a given composition (c_{A}). It is related to $I(x)$ in a simple manner, namely

$$W(s, c_{\text{A}}) = g_2 s\mu I(x) \tag{10}$$

where g_2 is a suitable normalization factor. The compositional distribution histogram reports, instead, the weight $W(c_{\text{A}})$ of chains which possess given composition (c_{A}) and it is therefore obtained by summation over all chain sizes, namely

$$W(c_{\text{A}}) = \sum W(s, c_{\text{A}}) \tag{11}$$

where the summation is over s and it goes from one to infinity.

Eqs. (1)–(11) were implemented in a computer program called COPOFRAC which is written in Quickbasic and runs on PC. COPOFRAC accepts as input the fractionation conditions, namely a_0, b_0, b_1, V_0, V_1, the parameters which describe the heterogeneity of the copolymer sample, $\bar{M}_{\text{n}}$, $\bar{M}_{\text{w}}$, d_0, d_1, d_2 and it gives as output the mass spectrum and the ^{1}H NMR spectrum of each fraction. COPOFRAC also gives as output the bivariate distribution (see Eq. (10)) and the compositional distribution histogram (see Eq. (11)). Furthermore, it allows to predict what happens when two (or more) SEC fractions obtained from a random copolymer are mixed together (see Eq. (9)).

3. Experimental section

3.1. Materials

Copolymer samples MB25 and MB41 were purchased from Rohm and Haas (Frankfurt, Germany). They are random copolymer samples of methylmethacrylate (MMA) and butylacrylate (BA). Sample MB25 was polymerized in ethylacetat using azobisdiethyl isobutyrate as initiator. The monomers conversion amounts to about 4% and the average molar fraction of MMA in the copolymer is 0.25, as measured by NMR. Sample MB41 was polymerized in ethylacetate using *tert*-butylperpivalate as initiator. The monomers conversion amounts to about 100% and the average molar fraction of MMA in the copolymer is 0.41, as measured by NMR.

Copolymer samples SH91 and SH78 were purchased from MPDAJAC (Feasterville, PA). They are random copolymer samples of styrene (St) and maleic-anhydride (MAH) obtained at high conversion from solution polymerization using AIBN (*N*,*N'*-azo-bisisobutyronitrile) as the initiator. The average molar fraction of St in the copolymer is 0.78 for sample SH78 and 0.91 for sample SH91.

Copolymer sample M30 was purchased from Polyscience (Feasterville, PA). It is a random copolymer sample containing units of styrene (St) and of methylmethacrylate (MMA) obtained at high conversion. The average molar fraction of MMA in the copolymer is 0.30.

3.2. SEC fractionation

The analyses were performed on a Polymer Lab apparatus, equipped with five ultraStyragel Waters columns (in the order 1000, 500, 10,000, 100, and 100,000 Å pore size) attached in series, using a Polymer Lab differential refractometer. The solvent was THF or $CHCl_3$ the flow rate was 1 ml/min and 60 μl of polymeric solution (15 mg/ml) were injected. Normally 50 fractions of 0.2 ml were collected. In the case of sample M30, four different fractionation experiments were performed and 50 fractions of 0.2 ml, 25 fractions of 0.4 ml, 15 fractions of 0.8 ml, 15 fractions of 1 ml were collected.

The chromatogram was calibrated using the result of the analysis of MALDI–TOF spectra of selected fractions (see Tables 1–3). The average molar masses ($\bar{M}_n$ and $\bar{M}_w$) of the copolymer were measured using the Caliber software distributed by Polymer Lab. The type of calibration selected by us was a ‘narrow standards’; the calibration function was ‘polynomial of order 1’ and the calculation method was ‘area based’.

3.3. NMR measurements

The ^{1}H NMR spectra of the SEC fractions 34, 37, 40, 43, 45, 50 and 53 of sample M30 were recorded on a Bruker A-CF 200 spectrometer. All the other NMR analyses were performed on a Varian Unity Inova 500 spectrometer at room temperature using $CDCl_3$ as a solvent and tetramethylsilane as internal standard. The ^{1}H NMR spectra were acquired with the following acquisition parameters: spectral width 4722.3 Hz, 131,072 data points, 700 accumulations and an acquisition time of 3.47 s.

DEPT subspectra of SH91 and SH78 were generated using the following combination of the π/4 and 3π/4 experiments: $CH_2 = (\pi/4) + 1.15(3\pi/4)$. Experiments were performed using a recycle time of 2 s and a *J*-modulation time of 3.7 ms. ^{1}H and ^{13}C π/2 pulse times were 22 and 15 μs, respectively.

Table 1
SEC/MALDI and SEC/NMR data for copolymer sample MB41

Fraction	Ve[a]	C_{MMA}[b]	$\bar{M}_n$[c]	$\bar{M}_w$[c]	*D*[c]	Weight[d]
30	27.75		173,000	175,000	1.01	0.157
31	28.25		133,000	135,000	1.03	0.234
36	29.11	0.39	89,000	90,000	1.01	0.282
40	30.01		72,000	73,500	1.02	0.298
41	30.25	0.35	64,000	67,000	1.04	0.291
42	30.52		55,000	57,000	1.04	0.274
43	30.75		50,000	51,500	1.03	0.252
44	31.01		45,000	47,700	1.06	0.223
45	31.25		39,000	41,400	1.06	0.194
46	31.52	0.42	34,000	35,400	1.04	0.164
47	31.75		31,000	32,860	1.06	0.139
48	32.01		28,000	29,400	1.05	0.107
49	32.25		25,000	26,000	1.04	0.099
50	32.51		23,000	23,920	1.04	0.084
51	32.75	0.42	21,000	21,840	1.04	0.073
54	33.51		17,000	17,900	1.05	0.046
56	34.01	0.65	13,500	13,800	1.02	0.036
58	34.51		11,000	11,400	1.03	0.031
61	35.25	0.78	7200	7800	1.08	0.024
62	35.51		6700	7250	1.08	0.022
65	36.25		5000	5400	1.08	0.018

[a] SEC elution volume (ml).

[b] Molar fraction of MMA in the copolymer fraction derived from NMR data using Eq. (12).

[c] $\bar{M}_n$, $\bar{M}_w$, and *D* of the SEC fraction as determined by MALDI.

[d] Weight fraction, as derived from the SEC trace.

Table 2
SEC/MALDI and SEC/NMR data for copolymer SH91

Fraction	Ve[a]	C_{St}[b]	$\bar{M}_n$[c]	$\bar{M}_w$[c]	*D*[c]	Weight[d]	Weight[e]
7	23.8	0.97				27	27
10	24.7	0.89				33	35
14	25.9	0.99				34	37
16	26.5		92,000	97,000	1.05	32	
18	27.1	0.78	71,000	74,000	1.04	29	31
20	27.7		54,000	57,000	1.05	26	
22	28.3	0.81	42,000	45,000	1.07	22	20
24	28.9		32,000	34,000	1.06	19	
25	29.2		28,000	29,000	1.04	18	
26	29.5	0.70	25,000	26,000	1.04	16	14
27	29.8		22,000	23,000	1.04	15	
28	30.1		19,000	20,000	1.05	13	
29	30.4	0.58	16,700	17,000	1.02	12	9
30	30.7		14,700	15,300	1.04	11	
31	31		12,900	13,500	1.05	10	
32	31.3	0.66	11,300	11,600	1.03	9	5
33	31.6		9900	10,400	1.05	8	
34	31.9		8700	9200	1.06	7	
35	32.2	0.81	6600	7800	1.18	7	5
36	32.5		5700	6900	1.21	6	
37	32.8		5900	6200	1.05	6	

[a] SEC elution volume (ml).

[b] Molar fraction of St in the copolymer fraction, derived from NMR data using Eq. (14).

[c] $\bar{M}_n$, $\bar{M}_w$, and *D* of the SEC fraction as determined by MALDI.

[d] Weight fraction, derived from the RI detector of the SEC apparatus without d*n*/d*c* correction.

[e] Weight fraction, derived from NMR data using Eq. (16).

Table 3
SEC/MALDI and SEC/NMR data for copolymer M30 ($\bar{M}_n$ and $\bar{M}_w$ determined from the doubly charged region of the MALDI spectrum)

Fraction	Ve[a]	F_{St}[b]	$\bar{M}_n$[c]	$\bar{M}_w$[c]	D[c]	Weight[d]	Weight[e]
16	23.3	0.53				2	0
18	23.7	0.57				3	1
20	24.1	0.59				6	4
22	24.5	0.61				10	8
24	24.9	0.62				15	13
26	25.3	0.63				22	20
28	25.7	0.64	180k	190k	1.05	30	26
31	26.3	0.65	129k	135k	1.04	44	40
34	26.9	0.66	100k	102k	1.02	58	54
37	27.5	0.67	77,500	81,500	1.05	69	66
40	28.1	0.68	60,000	62,000	1.03	75	73
43	28.7	0.69	46,500	48,000	1.03	74	77
45	29.1	0.70	39,500	42,000	1.06	71	75
50	30.1	0.75	26,000	27,000	1.04	54	56
53	30.7	0.80	20,000	21,923	1.09	41	43
55	31.1	0.84	17,000	18,384	1.08	33	36
57	31.5	0.89	14,500	15,273	1.05	27	30

[a] SEC elution volume (ml).

[b] Molar fraction of St in the copolymer fraction derived from NMR data using Eq. (17).

[c] $\bar{M}_n$, $\bar{M}_w$, and D of the SEC fraction as determined by MALDI.

[d] Weight fraction, derived from the RI detector of the SEC apparatus.

[e] Weight fraction, derived from NMR data using Eq. (18).

3.4. NMR calculations

The ^{1}H NMR spectra for random MMA–BA copolymer MB41 yielded an estimate of the copolymer composition [29]. The molar fraction of MMA in the copolymer, F_{MMA} is given by

$$F_{MMA}(x) = 1/[1 + 1.5A_{MMA}/A_{BA}] \tag{12}$$

where A_{MMA} is the area of the region 4.16–3.92 ppm, corresponding to MMA units, and A_{BA} the area in the region 3.66–3.51 ppm, corresponding to butylacrylate units. The variance of compositional distribution for MMA, σ^2, was computed from the abundances, I_{MMM}, $I_{MMB+BMM}$, I_{BMB}, of the M-centered triads using the formula

$$\sigma^2 = \{(3I_{MMM} - k)^2 + (2I_{MMB+BMM} - k)^2 + (I_{BMB} - k)^2\}/3 \tag{13}$$

where $k = F_{MMA}DP_n$, DP_n is the number-average degree of polymerization. The abundances of the M-centered triads are related to the areas under the MMM, MMB + BMM, and BMB resonances [30] (the coisotactity factor is 0.40).

The ^{1}H NMR spectra for random St–MAH copolymers yielded an estimate of the copolymer composition [30], which was determined using the formula

$$F_{st} = 0.2I_{arom}(0.5I_{alif} - 0.1I_{arom})^{-1} \tag{14}$$

where F_{st} is the molar fraction of styrene in the copolymer, I_{arom} is the area in the region 6–8 ppm, corresponding to styrene units and I_{alif} is the area in the region 1–3 ppm. The overall variance of compositional distribution for styrene, σ^2, was computed from the areas under the SSS, SSM + MSS, and MSM resonances in the DEPT subspectra, I_{SSS}, $I_{SSM+MSS}$, I_{MSM}, using the formula

$$\sigma^2 = \{(3I_{SSS} - k)^2 + (2I_{SSM+MSS} - k)^2 + (I_{MSM} - k)^2\}/3 \tag{15}$$

where $k = F_{st}DP_n$, DP_n is the number-average degree of polymerization and F_{st} is the molar fraction of styrene in the copolymer.

The weight of copolymer in each fraction, w_{copo}, was determined using the formula

$$w_{copo} = (w_{TMS}/I_{TMS})\tau_1 I_{copo}/M_{fra} \tag{16}$$

where M_{fra} is the number-average molar mass of the fraction, I_{copo} is $I_{alif} + I_{arom}$, I_{TMS} is the area under the peak at 0 ppm, corresponding to tetramethylsilane (briefly TMS), w_{TMS} is the weight of TMS in the NMR tube (0.136 mg) and where τ_1 is a dimensionless constant equal to 1466.2, which arises from the fact that St and MAH repeat unit possess eight and two protons, respectively, whereas TMS possesses 16 protons.

The assignments for the resonances in the ^{1}H NMR spectra for random St–MMA copolymers have been reported [31]. The molar fraction of MMA in the copolymer, F_{MMA} and the weight of copolymer in each fraction, w_{copo}, was determined using the formulas

$$F_{MMA}(x) = 1/[1 + (3/5)A_{arom}/A_{mo}] \tag{17}$$

$$w_{copo}(x) = (w_{TMS}/A_{TMS})\tau_2(A_{arom} + A_{mo})/(\mu s_{mid}) \tag{18}$$

where A_{arom} is the area in the region 6–8 ppm, corresponding to styrene units and A_{mo} is the area in the region 1–3 ppm due to methoxy protons of MMA units, A_{TMS} is the area under the peak at 0 ppm, corresponding to tetramethylsilane (briefly TMS), w_{TMS} is the weight of TMS in the NMR tube and τ_2 is a numerical factor which is equal to 916. The overall variance of compositional distribution for styrene, σ^2, for this copolymer sample was computed from the areas under the styrene-centered triads.

3.5. MALDI–TOF mass spectra

A BRUKER REFLEX mass spectrometer was used to obtain the matrix assisted laser desorption/ionization time of flight mass spectra. The spectrometer is equipped with a Nitrogen laser (337 nm, 5 ns), a flash ADC (time base of 4 ns) and a HIMAS detector. The laser irradiance was slightly above threshold (ca. 10^6 W/cm^2). Ions below *m/z* 350 were removed with pulsed deflection and 100 transients were summed. The MALDI mass spectra of the SEC fractions were processed with the XMASS program from Bruker. The program uses mass spectral intensities to compute the quantities known as most-probable molar mass, number-average molar mass, weight-average molar mass, and polydispersity index (denoted as Mp, $\bar{M}_n$, $\bar{M}_w$ and D, respectively) of each selected fraction.

A small amount (0.02 ml) of the chromatographic eluate in each fraction were added to 0.02 ml of a 0.7 M solution of 2-(4-hydroxyphenylazo)-benzoic acid (HABA) matrix. Probe tips were loaded with 1–2 μl of the resulting solution and the solvent was slowly evaporated.

4. Results and discussion

Copolymer sample MB41 is a high conversion (100%) random copolymer of methylmethacrylate (MMA) and butylacrylate (BA) produced by radical initiation. The sample was injected into the SEC apparatus, and about 40 fractions were collected. Several SEC fractions were then subjected to off-line MALDI and NMR analysis, respectively.

The SEC fractions analyzed by MALDI–TOF yielded excellent spectra with narrow distributions up to high molar masses (170,000 Da), and the mass spectra of these nearly monodisperse samples allowed the computation of reliable values of the molar masses corresponding to the fractions. The log(M) values of the fractions showed a linear correlation with the elution volume of each fraction and allowed the calibration of the SEC trace against MM, and the calibrated SEC trace could then be used to compute average molar mass and dispersion of the unfractionated copolymer ($\bar{M}_w = 91,000$, $\bar{M}_n = 43,000$, $D = 2.1$).

The SEC fractions were also analyzed by 500 MHz ^{1}H NMR and the signal-to-noise ratio was acceptable for all spectra (it never fell below 13:1). Peaks in the region between 4.2 and 3.5 ppm were considered and more specifically, the region 4.16–3.92 ppm, corresponding to MMA units, and the region 3.66–3.51 ppm, corresponding to butylacrylate units. The copolymer composition of each fraction was determined using Eq. (9).

Table 1 reports a summary of the MALDI and NMR results. From data in Table 1, it can be seen that the composition varies, and the fractions taken in the SEC region close to the peak elution volumes possess compositional values close to the average ones ($F_{\mathrm{MMA}} = 0.41$). At higher masses, the composition takes values up to 65% BA. At low masses, instead, the macromolecular chains are rich in MMA (about 78%, Table 1).

Peaks belonging to the ^{1}H NMR spectrum in the 3.66–3.51 ppm region turned out to be well resolved. For instance, Fig. 1 reports a section (with the cited region) of the 500 MHz ^{1}H NMR spectrum for fractions 46 and 51, along with the spectrum of the unfractionated sample. Peaks in the cited region can be assigned to the M-centered triads and, from the inspection of the figure, it can be seen that the intensities of the peaks are different. This implies that the relative abundances of M-centered triads in fraction 46 are different from the corresponding abundances in fraction 51 and in the unfractionated copolymer.

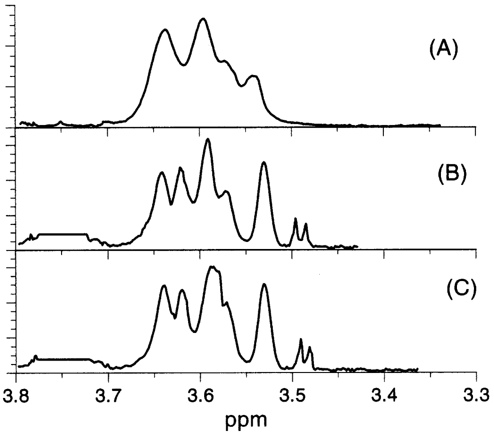

Fig. 1. Expansion of the 3.3–3.8 ppm region of the 500 MHz ^{1}H NMR spectrum for sample MB41. The unfractionated sample (A) fraction 46 (B) fraction 51 (C).

Inserting peak intensities in Eq. (11), the variance of the compositional distribution (σ^2) was derived. The variance for fractions 46 and 51 turned out to be slightly lower than the variance for the unfractionated copolymer, but the order of magnitude is the same. This result does not come unexpected, since MALDI–TOF data on a series of high-conversion MMA–BA random copolymers indicate that σ^2 for SEC fractions is virtually identical to σ^2 for the unfractionated copolymer [23].

The computer program COPOFRAC was used to generate the compositional distribution histogram for sample MB41 and the result is shown in Fig. 2. It can be seen that the weight is large in the region 0.2–0.5, that the tallest value is for $F_{\mathrm{MMA}} = 0.38$, which falls very close to the average ($F_{\mathrm{MMA}} = 0.41$).

Copolymer samples SH91 and SH78 are random copolymer samples of styrene (St) and maleic-anhydride (MAH) obtained at high conversion from solution polymerization using AIBN (N,N'-azo-bisisobutyronitrile) as the initiator.

The ^{13}C—125 MHz spectrum of samples SH91 and SH78 (omitted for brevity) does not yield sequence information because the signals due to methine and methylene

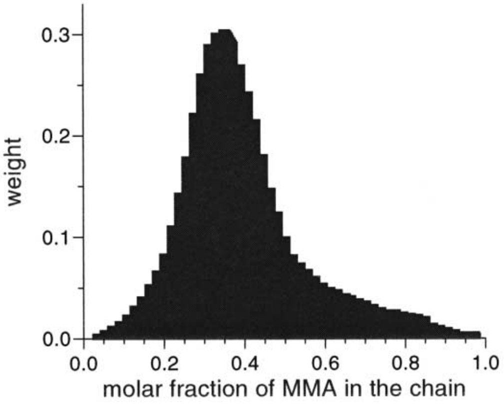

Fig. 2. Compositional distribution for sample MB41.

carbons are partially overlapped. However, sequence information can be obtained from DEPT experiments, by recording the spectra at π/4 and at 3π/4 and then combining them together. The areas under the SSS, SSM + MSS, and MSM resonances are 0.32, 0.50 and 0.18, respectively. In a similar manner, the areas under the SSS, SSM + MSS, and MSM resonances for sample SH91 were 0.39, 0.47 and 0.13, respectively. Inserting the three values for the SSS, SSM + MSS, and MSM resonances in Eq. (15), the overall variance of compositional distribution was found to be $\sigma^2 = 0.0844$ for sample SH78 and $\sigma^2 = 0.0676$ for sample SH91. The theoretical variances for SH78 and SH91 are $\sigma^2 = 0.015$ and $\sigma^2 = 0.0097$, respectively, which are lower than the former ones. Knowledge of the average composition and of the variance around the average may give some hints on the abundance of chains which possess a composition that differs from the average, although this is not sufficient to reconstruct the compositional distribution. For this reason, it is necessary to adopt a different approach, based on fractionation. Copolymers SH91 and SH78 were fractionated by SEC and 50–60 fractions were collected for each copolymer. The SEC fractions yield excellent MALDI–TOF spectra with narrow distributions (the polydispersity index is often smaller than 1.1, as reported in Table 2) up to high molar masses (up to 96 kDa). After that value, MALDI spectra became very weak and molar mass could not be estimated.

The copolymer fractions were also analyzed by ^{1}H NMR. The copolymer composition of each fraction was determined measuring the area of the regions 6–8 and 1–3 ppm and combining them together as depicted in Eq. (14). The composition values obtained by this procedure (see Table 2) imply that the molar fraction of styrene in both copolymer samples varies as the mass of the chain grows. In the case of sample SH91, the average molar fraction of styrene is in the range 0.58–0.99. Sample SH78 (data omitted for brevity) turned out to possess a more limited compositional heterogeneity since the average molar fraction of styrene is in the range 0.53–0.89.

The SEC data in Table 2 indicate that the copolymer fractions have a composition close to alternating (50%) for a molar mass of about 17–19 kDa. This molar mass corresponds to the kinetic chain length of the alternating copolymer and that lower and higher molar masses are subjected to higher percent of styrene inclusion.

Thereafter, ^{1}H NMR data was used to measure the amount of copolymer contained in each fraction by measuring I_{TMS} (the area under the peak at 0 ppm, corresponding to tetramethylsilane) and combining it with the quantities I_{arom} and I_{alif} defined in Eq. (16). The resulting weight values are reported in Table 2 (column 8). In order to measure the amount of copolymer, one may rely on the SEC detector, which is a differential refractometer, and assume that the detector's response reflects the amount of copolymer. Table 2 (column 7) reports the amount of copolymer estimated using the latter method. There are some discrepancies between the cited (RI) values and the values obtained by ^{1}H NMR. The discrepancies between the two measurements are often small; however, in some cases they become large and cannot be neglected. For instance, the two values for the amount of copolymer in fraction 36 are 17 and 26, which implies a difference larger than 30%. This difference is due to the fact that we are dealing with compositionally heterogeneous copolymers; the response to styrene units and to maleic-anhydride units may be different and the refractometer's response is therefore unreliable. A UV detector is commonly added to the apparatus and used to correct for the different values of the quantity d*n*/d*c* for the two monomers. However the time-lag estimation between the two detectors is cumbersome [1–6].

The average molar mass and dispersion of the unfractionated copolymers were then computed. The software for such calculations needs the calibration lines for the two samples and the abundances. The calibration data was taken from Table 2 (column 5), which reports the MALDI–TOF analysis of each fraction. The result was $\bar{M}_{\mathrm{n}} = 151{,}000$, $\bar{M}_{\mathrm{w}} = 345{,}000$, $D = 2.28$.

Computer program COPOFRAC was used to generate the compositional distribution histogram for sample SH91 and the result is shown in Fig. 3. This histogram differs from the preceding one (Fig. 2) in that it is almost flat, especially in the region 0.7–0.9, which implies that the compositional distribution is broad. This agrees with the results of the DEPT–NMR experiment, which indicated that the overall variance of compositional distribution for sample SH78 is large. These results can be understood since the MAH monomer is consumed in the first part of the copolymer forming reaction. Therefore in the SH91 copolymer sample, when the conversion is above 50%, the compositional distribution becomes very rich in styrene units, showing a neat maximum at 95% in styrene units (Fig. 3).

Copolymer sample M30 is a random copolymer with units of styrene (St) and methylmethacrylate (MMA) obtained at high conversion. The copolymer was injected in the SEC apparatus, the SEC fractions were collected and analyzed by ^{1}H NMR and MALDI. Fig. 4A reports the

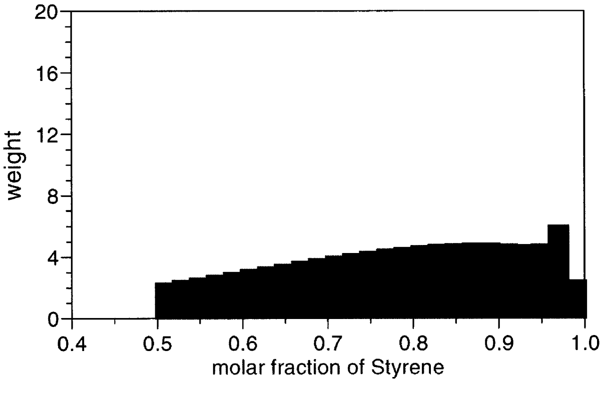

Fig. 3. Compositional distribution for sample SH91.

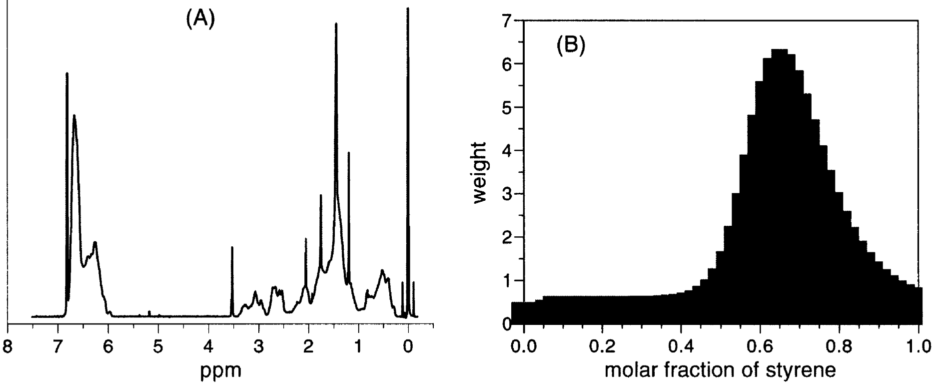

Fig. 4. SEC fractionation for sample M30. The 250 MHz NMR spectrum of fraction 34 (A) and compositional distribution for the sample (B).

200 MHz ^{1}H NMR spectrum of fraction 34. It can be seen that the signal-to-noise ratio is acceptable and this demonstrates that off-line SEC–NMR can be performed using medium–low field magnets (200 MHz), a very attractive feature indeed!

Table 3 reports a summary of the MALDI and NMR results. From data in Table 3, it can be seen that the composition varies dramatically as the molar mass increases. Specifically, the molar fraction of styrene is very high at low masses (14,500), then it falls steadily (in an almost linear manner) and it reaches the value of 0.53 at high mass.

SEC fractions of M30 yielded acceptable MALDI–TOF mass spectra (omitted for brevity) with a peculiar feature, namely strong signals due to doubly-charged ions. The calibration data were used to compute the molar mass averages, which turned out to be $\bar{M}_n = 99,000$, $\bar{M}_w = 240,000$, $D = 2.4$.

Computer program COPOFRAC was used to generate the compositional distribution histogram for sample M30 and the result is shown in Fig. 4B. This histogram differs from the preceding ones (see Figs. 2 and 3) since there is a plateau in the styrene-poor region, which originates from long MMA–rich chains. When the molar fraction of styrene increases from 0.4 to 0.65, the weight increases thereafter it bends downwards.

The amount of copolymer in each fraction was computed using Eq. (18). Fig. 5 reports the SEC traces of the M30 sample: before correction (full line) after correction (dotted line). SEC chromatogram as resulting from the RI detector is misleading, since it takes its tallest value about 1 ml earlier, which corresponds to a 33% error in the determination of $\bar{M}_n$ and $\bar{M}_w$.

In the case of sample M30, four different fractionation experiments were performed and 50 fractions of 0.2 ml, 25 fractions of 0.4 ml, 15 fractions of 0.8 ml, 15 fractions of 1 ml were collected. The goal of these experiments is to find the optimal conditions, namely the largest volume of the fraction where the above-mentioned loss of accuracy is small and to collect experimental data which can be used to test the model for copolymers obtained by SEC fractionation described in the Section 2.

Fig. 6A–D reports the MALDI–TOF mass spectra of the fractions collected around 29 ml. The four spectra are bell-shaped, the tallest molar mass is about 46,000) and it is quite apparent that the spectrum becomes broader as the volume of the fraction grows. The MALDI–TOF mass spectra of the fractions collected around 30 ml (data omitted for brevity) are bell-shaped too and the tallest molar mass is about 26,000.

Computer program COPOFRAC was used to predict the polydispersity index (D) versus V_l (the volume of the fraction) for this experiment. Fig. 6E (full line) reports the result of the calculation and it can be seen that D scales in a non-linear manner (this implies that Eq. (8) does not possess an analytical solution) and that when V_l is larger than 0.6 ml, D becomes unacceptably large. On the other hand, when V_l is smaller than 0.6 ml, D is unacceptable and thus these represent the optimal conditions. Fig. 6F (full line) reports the result of a similar calculation for fractions collected around

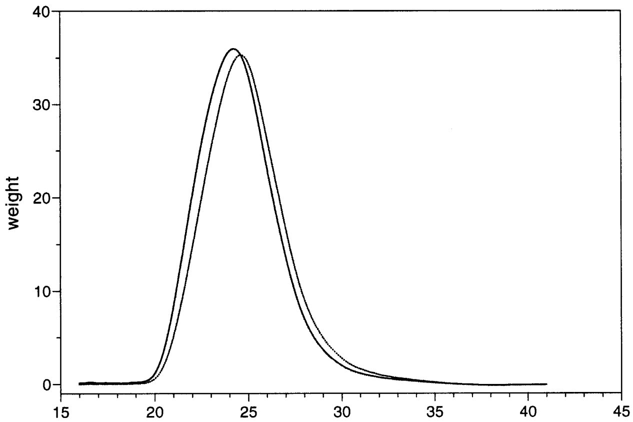

Fig. 5. SEC traces of the M30 sample: before correction (full line) after correction (dotted line).

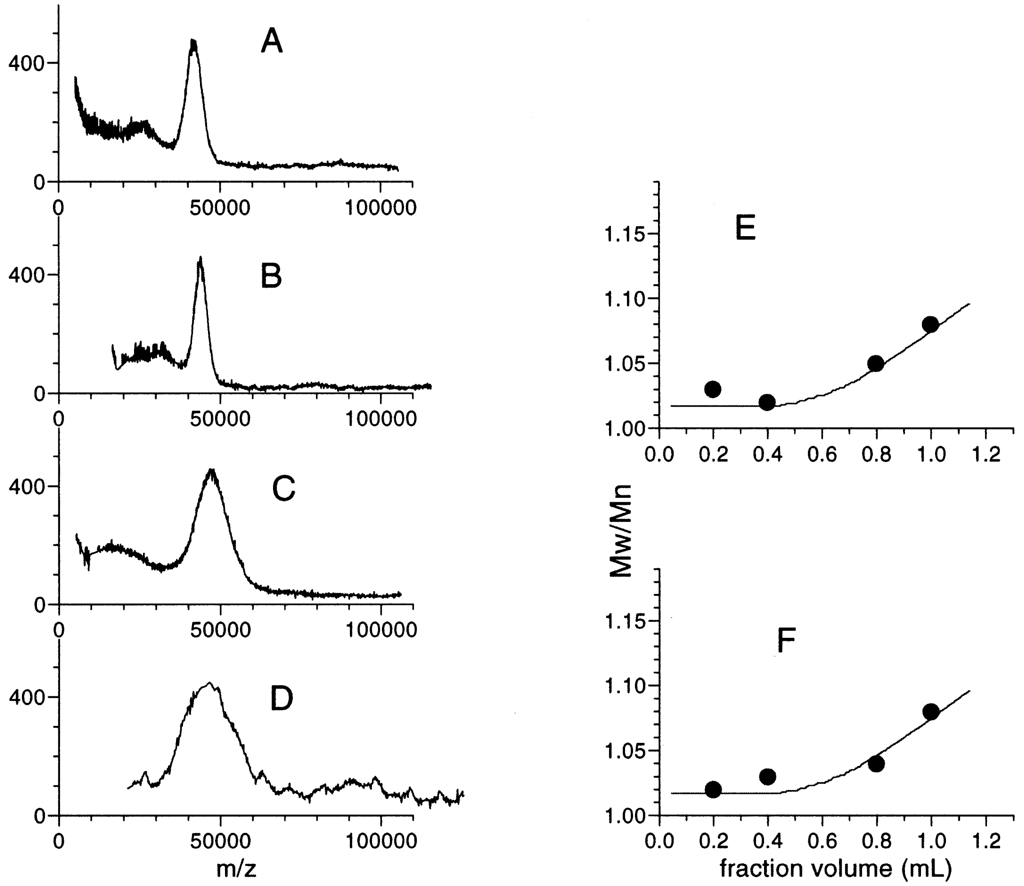

Fig. 6. MALDI of fractions of sample M30 collected in four different experiments. The volume of the fraction is 0.2 ml (A), 0.4 ml (B) 0.8 ml (C), 1.0 ml (D). The $\bar{M}_w/\bar{M}_n$ ratio of the fractions is also displayed for fractions collected around 29 ml (E) and around 30 ml (F).

30 ml. Again, the optimal conditions are V_1 smaller than 0.6 ml. Fig. 6e (points) reports the experimental data for D of fractions collected around 29 ml whereas Fig. 6f (points) reports analogous for fractions collected around 30 ml. These data allow a comparison between theory and experiment and the agreement is good, demonstrating the validity of the model proposed.

Computer program COPOFRAC was used to predict the changes in composition as the volume of the fraction grows and, more specifically, the difference between the composition of the SEC fraction and the composition of the unfractionated copolymer, which is related to the quantity ϕ defined in Eq. (6). Fig. 7A (full line) reports the result of the calculation and it can be seen that ϕ varies linearly with λ (which is defined by $\lambda = \log(V_1)$, see Eq. (7)) and that when V_1 becomes larger and larger, the composition of the SEC fraction becomes virtually coincident with that of the unfractionated copolymer (the difference drops to zero and the logarithm tends to minus infinity). Fig. 7A (points) reports the experimental data for ϕ of fractions collected around 29 ml, plus an additional point, obtained from Eq. (4) (which gives the composition when SEC fraction x is mixed together with SEC fraction y). Data in Fig. 7A can be used to compare predicted and experimental ϕ values. There is a fair agreement, and the linear scaling of ϕ with λ is clearly confirmed.

An interesting feature of the model developed for copolymers obtained by SEC fractionation is that it predicts that a compositionally homogeneous copolymer can yield a compositionally inhomogeneous fraction. This is caused by the 'mass coelution' effect discussed in Section 1. Specifically, when the quantities ρ_1 and ρ_2 in (Eq. (5)) are large, the fraction possesses a drift and is therefore compositionally inhomogeneous.

Computer program COPOFRAC was used to predict how the composition varies with chain length q in the case of SEC fractionation of a copolyester with units of butileneadipate and butilenesebacate [22] and more specifically the molar fraction of butilene-adipate units for some SEC fractions. Fig. 7B (full line) reports the result of the calculation for fraction 26 and it possesses three most evident features, namely the curve is more or less straight (a line), the molar fraction of butilene-adipate units increases with chain size and the slope is small, but certainly not negligible. The MALDI–TOF mass spectra of fraction 26 was reported elsewhere [22] along with a thorough tabulation of

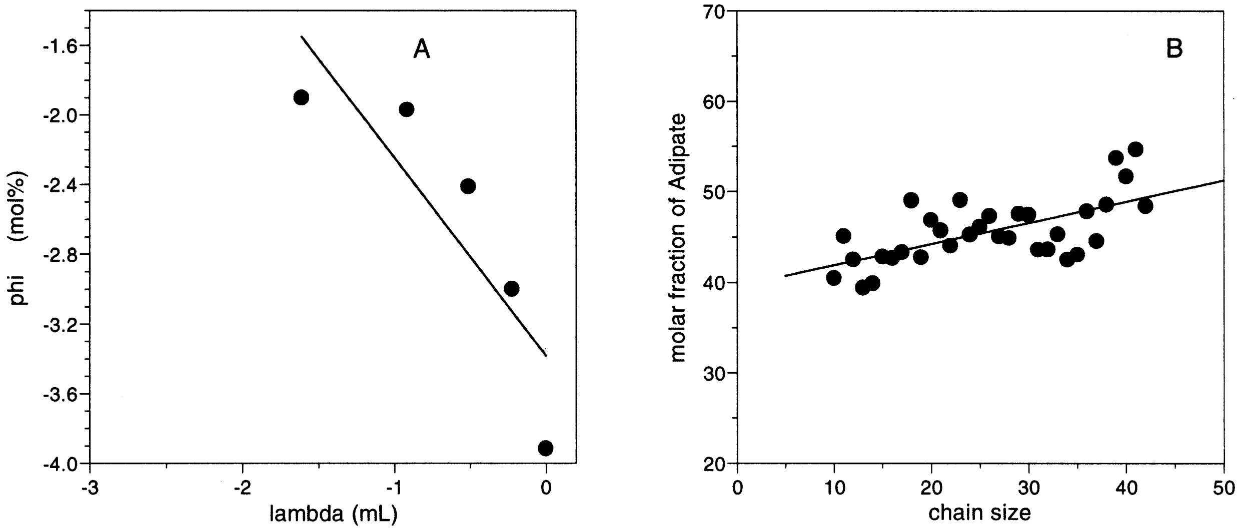

Fig. 7. Comparison between theory (full lines) and experiment (points). ϕ versus λ in Eq. (5) (A) and molar fraction of adipate versus chain size (B).

mass spectral intensities. Using the intensities, the molar fraction of butileneadipate units for each chain size was derived as reported in Fig. 7B (points).

It can be seen that point are somehow scattered, but the fact that the molar fraction of butileneadipate units increases with chain size stems out clearly. This implies that the fraction is compositionally inhomogeneous and it demonstrates that the prediction of the model is correct.

5. Conclusions

An method for full copolymer characterization is presented, which employs off-line SEC–NMR, SEC–MALDI. A series of examples of application on copolymers reacted at high conversion are discussed. Data are shown which demonstrate that off-line SEC–NMR, SEC–MALDI can be performed using medium field magnets (200 MHz), a very attractive feature since it allows the use of affordable equipment.

A theoretical model is developed which allows to predict the mass spectrum and the ^{1}H NMR spectrum of each SEC fraction. It also generates the bivariate distribution (see Eq. (10)) and the compositional distribution histogram (see Eq. (11)) and predicts what happens when two (or more) SEC fractions are mixed together.[1]

Acknowledgements

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[1] There is a plan to modify the WEB page of our institute (CNR-ICTMP-CATANIA) to add the listing of computer program COPOFRAC.

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