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Journal of Chromatography A, 1055 (2004) 123–133
JOURNAL OF CHROMATOGRAPHY A
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Separation and characterization of functional poly(*n*-butyl acrylate) by critical liquid chromatography

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Received 26 May 2004; received in revised form 27 August 2004; accepted 30 August 2004

Abstract

The separation of functional poly(*n*-butyl acrylate) (PnBA) polymers based on the number of end-groups under critical liquid chromatography (LC) conditions has been studied using a bare-silica column. The (near-) critical solvent compositions for non-, mono-, and difunctional (telechelic) carboxyl-PnBAs were determined in normal-phase LC, using mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying composition. Some formic or acetic acid had to be added to the mobile phase to elute PnBA polymers with carboxyl end-groups. The critical solvent compositions obtained were not exactly the same for non-, mono-, and difunctional PnBA polymers. These were unusual experimental observation, but they were in agreement with theoretic predictions. Nevertheless, low-molecular-mass PnBA samples were successfully separated according to the carboxyl functionality at (near-) critical conditions. With the aid of mass spectrometry (MS), the (near-) critical separation of low-molecular-mass PnBA polymers was confirmed to be mainly based on the carboxyl functionality. Calibration curves for evaporative light-scattering detection (ELSD) were used for quantitative analysis of carboxyl-functional PnBA polymers. The results proved that nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved for telechelic PnBAs prepared by one-step reversible addition–fragmentation chain-transfer (RAFT) polymerization of PnBA.

Keywords: Critical liquid chromatography; Telechelic polymers; RAFT; Functionality; Carboxylic acid end-group

1. Introduction

The matrix of polymer coatings are generally made of two precursor components, which form during processing a three-dimensional molecular network. The completeness of these network structures, the (average) distance between the cross-links and the amount of dangling ends influence strongly the T_g of the coating and the final material properties, such as hardness, scratch resistance and durability. Unfortunately, limited knowledge is available about the relation between these network characteristics and the material properties of the final coatings and therefore, in practice, coatings with an optimum set of such properties can hardly be made yet. For a better understanding of the structure–property relationships of such coatings, short chain highly pure polymers or oligomers with well-defined molecular masses and low polydispersities and with well-characterized amount and type of reactive groups are required. To study the influence of dangling ends in these networks, oligomers/polymers with monofunctional as well as

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doi:10.1016/j.chroma.2004.08.136

bifunctional reactive end-groups are required. For example, highly pure, linear telechelic poly(meth)acrylates, bearing hydroxyl or carboxyl end-groups and corresponding monofunctional polymers are very attractive candidates for such a study. However, it is difficult to produce these and other telechelic well-defined polymers with the desired end-groups by radical conventional polymerization methods. The functional polymers in this work have one or two hydroxyl or carboxyl end-groups and their functionality means the number of hydroxyl or carboxyl end-groups.

In the past 20 years, numerous research groups have devoted their efforts towards the control of radical-polymerization processes. The most important methodologies include nitroxide-mediated radical polymerization [1], atom-transfer radical polymerization (ATRP) [2], and, most recently, reversible addition–fragmentation chain-transfer (RAFT) polymerization [3]. Among these, RAFT may arguably have the greatest commercial impact, because the process only involves organic substances and because it works very well with most vinyl monomers, including acrylic acid [4]. RAFT can be employed in many polymerization processes (e.g. bulk, solution, suspension, emulsion polymerization [5]).

Therefore, we have selected RAFT polymerization to prepare linear α,ω-functional polymers (commonly known as telechelic polymers) with either hydroxyl or carboxyl end-groups and low polydispersities. Lima et al. [6] have described various reaction schemes and procedures to synthesize well-defined telechelic poly(meth)acrylates using functionalized initiators and RAFT agents. Their work has resulted in polymers with predictable molecular masses and low polydispersities. Ideally, linear telechelic polymers have a functionality of two. It is a serious challenge to approach this limit, mainly because of side reactions inherent to growing radicals, such as termination by bimolecular combination or disproportionation [7]. Moreover, chain transfer to solvent molecules or to monomers will always occur. Finally, in some cases, post-polymerization modifications are necessary. Experience with macromolecular reactions indicates that hydroxy-endcapping reactions do not proceed with full conversion [7], and that they are accompanied by side reactions [6,8].

The development and optimization of procedures for the synthesis of well-defined functional polymers is vitally dependent on effective analytical methods to determine the functionality-type distribution (FTD) and the molecular-mass distribution (MWD). The traditional analytical techniques, such as infrared absorption spectroscopy (IR), ultraviolet spectroscopy (UV), nuclear magnetic resonance spectroscopy (NMR), and specific titrations of –OH or –COOH groups, are inadequate for this purpose, because only the *average* functionality can be measured, and not the FTD. Mass spectrometry (MS) can provide good qualitative information on polymer end-groups, but poor quantitative results. This is because of the variation in the ionization efficiency for different functional polymers. Also mass-discrimination and ion-suppression effects are encountered in polymer MS [9]. To determine accurate FTDs and specific MWDs for molecules of a given functionality, the functional polymers must be separated based on the number of end-groups.

The application of critical liquid chromatography (LC) in polymer science and industry is still new and challenging [10–17]. In principle, this technique can be used to separate polymers exclusively according to the number of functional groups, for example, into non-, mono-, and difunctional polymers [18–21]. However, critical conditions do not necessarily provide good functionality-based separations [10]. In a previous study [22], we have established robust critical LC conditions for the separation of hydroxyl-functional poly(methyl methacrylate) (PMMA) samples. With the aid of mass spectrometry, separation was confirmed [8] to be mainly based on the number of hydroxyl groups present in the low-molecular-mass RAFT polymers, either with or without end-group modification. In this work, a similar strategy is employed for a different polymer with different functional end-groups. As model polymers for this study, poly(*n*-butyl acrylate) (PnBA) with COOH end-groups were synthesized by using one-step RAFT polymerization, according to the procedure described by Lai et al. [4]. The resulting polymers had to be separated based on the number of end-groups. Various possible (near-) critical solvent compositions for PnBA were studied in normal-phase LC. Ternary mixtures of acetonitrile, dichloromethane, and acetic (or formic) acid were explored. The temperature was also varied. Subsequently, MS was used to identify the repeating units and, especially, the end-groups of the fractionated polymers.

2. Experimental

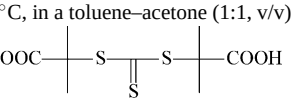
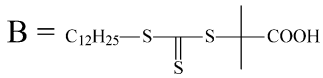
2.1. Chemicals

Dichloromethane (DCM), acetonitrile (ACN) (both HPLC grade, from Rathburn Chemicals (Walkerburn, UK), formic acid (FA, analytical-reagent grade, Merck, Darmstadt, Germany), and acetic acid (HAc, analytical-reagent grade, Acros Organics, Geel, Belgium) were used without further purification. Non-functional poly(*n*-butyl acrylate) samples were synthesized by reversible addition–fragmentation chain-transfer (RAFT) polymerization ("RAFT polymers") using 2,2′-azobisisobutyronitrile (AIBN, Merck) as initiator and a non-COOH-functional RAFT chain-transfer agent (2-cyanoprop-2-yl-dithiobenzoate, RAFT-AIBN). PnBAs with one COOH group were synthesized by RAFT polymerization using AIBN or carboxyl-terminated azo-initiator 4,4′-azobis(4-cyanovaleric acid) (ACVA, Aldrich, Milwaukee, WI, USA) as the initiator and a monocarboxyl-terminated trithiocarbonate derivative, *S*-1-dodecyl-*S*′-(α,α′-dimethyl-α″-acetic acid)trithiocarbonate, as the RAFT chain-transfer agent. Linear PnBAs with two COOH groups were synthesized by RAFT polymerization using AIBN or ACVA as the initiator and a dicarboxyl-terminated trithiocarbon-

Table 1
Synthesis data of PnBA samples by RAFT polymerization[a]

Sample name	Initiator	CTA[b]	[RAFT]/[initiator]	Conversion (%)	$M_{n,exp}$ ($M_{n,th}$) ($\times 10^{-3}$)
VL068 (PnBA–2COOH 2510)	AIBN	A	20	99	2.23 (2.20)
VL103 (PnBA–2COOH 2400)	AIBN	A	8	95	2.09 (2.20)
VL123 (PnBA–2COOH 3200)	ACVA	A	8	97	2.83 (3.20)
VL127 (PnBA–2COOH 2500)	ACVA	A	8	99	2.26 (2.20)
VL131 (PnBA–COOH 2610)	ACVA	B	20	99	2.38 (2.20)

[a] All polymerizations were carried out at 80 °C, in a toluene–acetone (1:1, v/v) mixture under an argon atmosphere.

[b] CTA: RAFT chain-transfer agent. A =  B = 

ate, *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate, as the RAFT chain-transfer agent [6]. For the sake of clarity, some synthesis data are reported in Table 1. These data show the origin of structural differences between different functional RAFT-PnBA samples. The details of the synthetic procedures were described in [6]. The molecular masses and molecular-mass distributions were measured by size-exclusion chromatography (SEC) with a Waters (Milford, MA, USA) instrument consisting of a Waters model 510 pump and a model 410 differential refractometer (operated at 40 °C). Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 ml min^{-1}. The columns used were a PLgel guard column (5 μm particles) 50 mm × 7.5 mm, followed by two PLgel mixed-C (5 μm particles) 300 mm × 7.5 mm columns (Polymer Labs, Church Stretton, UK) kept in an oven at 40 °C. The calibration curve was prepared with polystyrene (PS) standards (molecular masses ranging from 580 to 7.1 × 10^6 g mol^{-1}) and the molecular masses were estimated based on the universal-calibration principle and Mark–Houwink parameters (PS: $K = 1.14 \times 10^{-4}$ dl g^{-1} and $a = 0.716$; PnBA: $K = 1.220 \times 10^{-4}$ dl g^{-1} and $a = 0.700$) [23]. The effect of the carboxyl end-groups on the Mark–Houwink parameters was neglected. All the PnBA samples used are summarized in Table 2. All the samples were dissolved in DCM unless stated otherwise.

2.2. Equipment

A Waters 2690 Alliance liquid chromatography system was used to perform the isocratic LC experiments. This HPLC instrument contained a built-in auto-injector with a sample loop allowing injection of variable sample volumes and it was equipped with a Waters 996 photodiode-array detection (DAD) system and a Sedex 55 evaporative light-scattering detection (ELSD; temperature, 62 °C, N_2 pressure, 2.2 bar) system. The mobile phase was prepared in situ using the solvent-mixing capability of the instrument. The formic or acetic acid was added in the form of a premixed solution of 10% (v/v) in DCM. All eluent compositions are given in % (v/v). The data collection and the data analysis were handled by Waters Millennium 3.2 software. The columns used (150 mm × 4.6 mm i.d.) were packed in the laboratory with Hypersil silica (3 μm particles; 100 Å pore size; Shandon, Runcorn, UK).

The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) measurements were performed with a Voyager-De Pro instrument (PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337 nm nitrogen laser. Spectra were acquired by summing the data obtained from 200 laser shots in the reflector mode. α-Cyano-4-hydroxycinnamic acid (about 20 mg ml^{-1} in THF) was used as the matrix. The concentration of the polymer sample was about 1 mg ml^{-1} in THF.

The electrospray ionization mass spectrometry (ESI-MS) experiments were carried out using a Finnigan LCQ Deca XP MAX ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Polymer samples were dissolved in an acetonitrile–methanol (1:1, v/v) mixture at a concentration of around 1 mg ml^{-1}. The flow rate of the sample introduced into the electrospray interface was 20 μl ml^{-1}. The electrospray-source voltage was 5.0 kV and the electrospray-

Table 2
PnBA samples used in this study

Sample name	M_n ($\times 10^{-3}$)	M_p ($\times 10^{-3}$)	PDI	Intended funtionality
PnBA 600[a]	NA[b]	6.0	NA[b]	0
PnBA 4690[a]	3.45	4.69	1.32	0
PnBA 7390[a]	4.24	7.39	1.53	0
PnBA 32 330[a]	19.28	32.33	1.49	0
PnBA–COOH 2610[c]	2.38	2.61	1.13	1
PnBA–COOH 13 260[c,d]	4.61	13.26	2.05	1
PnBA–COOH 19 090[c,d]	9.54	19.09	1.63	1
PnBA–2COOH 2400[d,e]	2.09	2.32	1.13	2
PnBA–2COOH 2500[e]	2.26	2.50	1.11	2
PnBA–2COOH 2510[d,e]	2.23	2.51	1.13	2
PnBA–2COOH 3200[e]	2.83	3.20	1.10	2
PnBA–2COOH 5540[d,e]	4.41	5.54	1.21	2
PnBA–2COOH 11 450[d,e]	8.64	11.45	1.22	2

The molecular mass (M_n, M_p) and polydispersity index (PDI) values were measured by SEC (calibration described in Section 2).

[a] Non-functional PnBA samples synthesized by reversible addition–fragmentation chain-transfer (RAFT) polymerization using a 2,2′-azobisisobutyronitrile (AIBN) initiator and a non-COOH-functional RAFT chain-transfer agent.

[b] Because the SEC peak overlaps with the solvent peak, the values of the molecular masses (M_n, M_w, and PDI) cannot be calculated accurately.

[c] PnBAs with one COOH group synthesized by RAFT polymerization. For synthetic procedures, see Section 2.

[d] AIBN initiator used in RAFT polymerization.

[e] PnBAs with two COOH groups synthesized by RAFT polymerization. For synthetic procedures, see Section 2.

source temperature was 275 °C. Mass spectra were scanned over the range *m/z* 500–4000 in the positive-ion mode. More than 100 scans were summed to produce the final spectrum.

ESI-MS–MS experiments were carried out on the selected precursor ions by low-energy collision-induced dissociation (CID) using helium as collision gas to obtain the fragmentation-ion spectrum.

3. Results and discussion

3.1. Critical conditions for PnBA

Our objective was to establish critical LC conditions to separate linear PnBA samples exclusively based on the number of carboxyl end-groups. In a previous study [22], robust critical LC conditions were established for the separation of hydroxyl-terminated PMMA samples using mixtures of ACN and DCM as the mobile phase. A bare-silica column was used, which gave rise to sufficiently strong interactions with the functional (hydroxyl) groups. DCM is a good solvent for PMMA and also for PnBA, but it is a weak eluent on a silica column [24]. These properties make it a good injection solvent in interactive LC, as breakthrough problems can be avoided [25]. In this study, all samples were dissolved in DCM unless stated otherwise. ACN is a more-polar solvent, which can desorb PnBA from the silica column. Therefore, these two solvents were selected to make up the mobile phase.

Following the method of Cools et al. [12], the obtained retention times for linear PnBA samples of varying molecular mass were plotted against the percentage of ACN (as shown in Fig. 1). The intersection point provides an indication of the critical solvent composition, at which the retention time of the polymer is independent of the molecular mass. The critical point for non-functional PnBA was found to be around 11% of ACN in DCM at 25 °C (see Table 3). However, the PnBA samples with one or two carboxyl end-groups were fully retained on the silica column under these conditions. Increasing the concentration of ACN in the mobile phase, even up to pure ACN did not result in elution of these samples, even though ACN is a good solvent for low-molecular-mass PnBAs with carboxyl end-groups. To overcome this problem, some formic acid or acetic acid, added in the form of a premixed solution of 10% (v/v) in DCM, had to be added to the mobile phase.

Fig. 1a–c show an example of the dependence of the retention time on the mobile-phase composition (percentage of ACN in DCM) at a constant concentration of 0.5% HAc and at 55 °C for non-functional PnBA, monofunctional PnBA–COOH, and difunctional PnBA–2COOH samples of varying molecular masses. The critical solvent compositions obtained were about 6.6, 5.8 and 5.5% (v/v) ACN in DCM for non-functional PnBA, monofunctional PnBA–COOH, and difunctional PnBA–2COOH polymers, respectively. The obtained critical points for various end-group series were not exactly the same. This was an unusual experimental observation, because, in principle, the critical composition for the PnBA backbone should not change with the end-groups. However, the observed differences could not be ascribed to experimental error. As shown in Table 3, a variation in the exact critical composition with variation in the end-groups present was also observed at other temperatures (25 and 40 °C). Gorbunov and Trathnigg [21] and Skvortsov and Fleer [26] ratiocinated from theory that the retention of difunctional polymers at the critical point for non-functional polymer depends on the molecular masses. They stated that the distribution coefficient of functional polymers could exceed unity and would decrease with the radius of gyration (molecular mass) if the interaction of end-groups with the stationary phase was strongly attractive. Our observations are in agreement with these predictions. At the critical point of non-functional PnBA (6.6% ACN), carboxyl end-groups show a strong attractive interaction with the stationary phase. The relative effect of this interaction will decrease with increasing molecular mass and, therefore, the retention of carboxyl-functional PnBA sample decreases. To reduce the effect of the molecular mass on the retention of carboxyl-functional PnBA samples, slightly less acetonitrile should be present in the mobile phase. The amount of acetonitrile required decreases with increasing number of end-groups. An example is shown in Fig. 1d, with 6% ACN and 0.5% HAc in DCM as the mobile phase at 55 °C. The retention for non-functional PnBA increased with increasing molecular mass. In contrast, the retention for monofunctional PnBA–COOH and difunctional PnBA–2COOH decreased with increasing molecular mass under the same conditions. It also can be seen from Fig. 1d that low-molecular-mass PnBA samples could be separated much more easily based on the number of carboxyl end-groups, than could high-molecular-mass PnBA samples.

It also can be seen in Fig. 1 that the critical conditions are rather sensitive to the exact eluent composition, which is in agreement with the data reported in [27]. Due to the uncertainty surrounding the exact location of the critical point

Table 3
Approximate critical ("near-critical") compositions for PnBA samples obtained at different acetic acid concentrations and at different column temperatures

Functionalities	Temperature (°C)	HAc (%)	ACN (%)	DCM (%)
Non-COOH	25	0	11	89
		0.5	8.9	90.6
		1.0	7.4	91.6
		3.0	6.0	91
	40	0.5	7.5	92
	55	0.5	6.6	92.9
Mono-COOH	25	0.5	6.9	92.6
	40	0.5	5.8	93.7
	55	0.5	5.8	93.7
Di-COOH	25	0.5	6.7	92.8
		1.0	6.1	92.9
		3.0	4.0	93
	40	0.5	5.7	93.8
	55	0.5	5.5	94

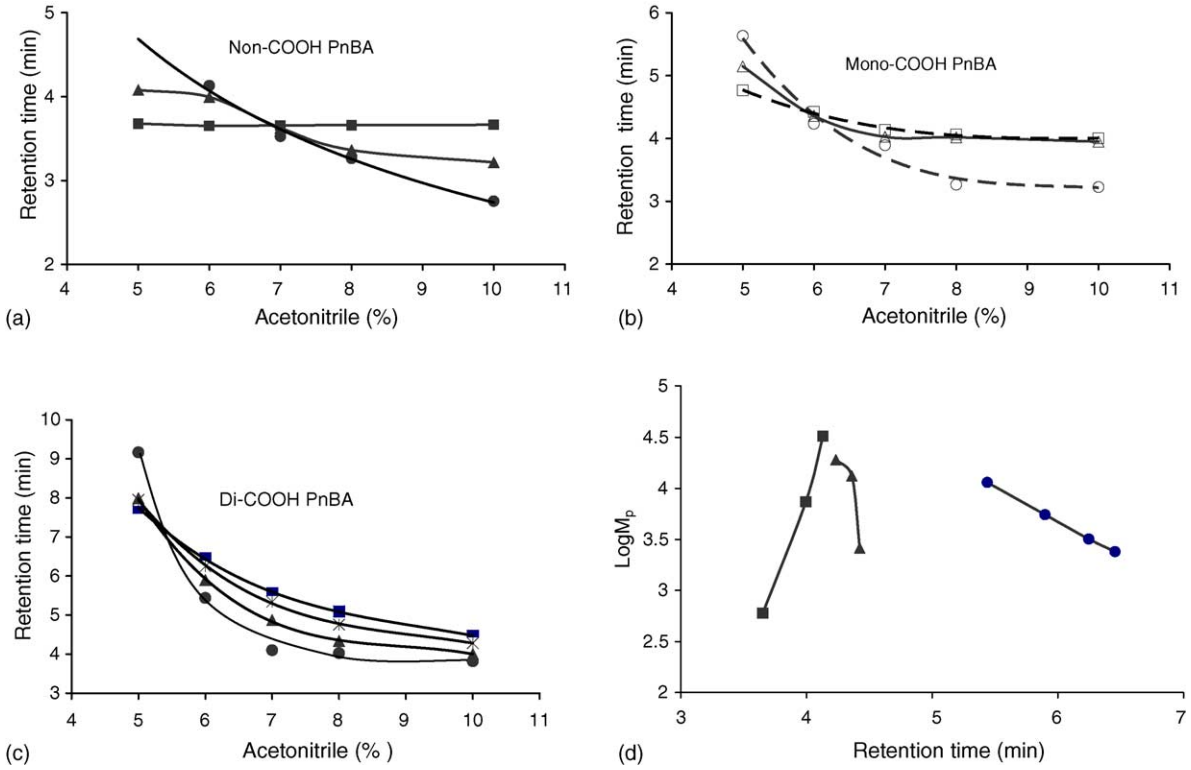


Fig. 1. Dependence of retention time on the mobile-phase composition (at 0.5% HAc) for PnBA samples with different molecular masses. (a) Non-functional PnBAs: (■) PnBA 600; (▲) PnBA 7390; (●) PnBA 32 330. (b) Monofunctional carboxyl-PnBAs: (□) PnBA–COOH 2610; (△) PnBA–COOH 13 260; (◯) PnBA–COOH 19 090. (c) Difunctional carboxyl-PnBAs: (■) PnBA–2COOH 2400; (✳) PnBA–2COOH 3200; (▲) PnBA–2COOH 5540; (●) PnBA–2COOH 11 450. (d) Molecular-mass effect on retention time for non-, mono-, and dicarboxyl-functional PnBAs under near-critical conditions (6% ACN and 0.5% HAc in DCM): (■) non-COOH; (▲) mono-COOH; (●) di-COOH. ELSD, laboratory-packed Hypersil silica column (150 mm × 4.6 mm i.d.; 3 μm particles; 100 Å pore size), flow rate, 0.5 ml min^{-1}; column temperature, 55 °C.

and the residual (slight) variation of retention with molecular mass, we speak of near-critical conditions in this paper. The present near-critical conditions are much less robust than the critical conditions reported previously for hydroxyl-functional PMMA [22]. From a combination of Fig. 1a–d, we conclude that a mobile phase containing about 6.0% ACN and 0.5% acetic acid in DCM can be used to separate the low-molecular-mass PnBA samples (up to 10 000) based on the number of carboxyl end-groups.

Fig. 2 shows representative ELSD chromatograms of PnBA samples of varying molecular masses and with different numbers of functional end-groups. The two different non-functional PnBA samples (with no COOH groups, molecular masses 4690 and 32 330) co-eluted. The two monofunctional polymers (with one COOH end-group, molecular masses 2610 and 13 260) had similar retention times, but were clearly separated from the non-functional PnBA samples. The expectedly telechelic (difunctional) samples (with two COOH end-groups, molecular masses 2500 and 11 450) were well separated from the monofunctional polymers. Note that there was a small amount of non-functional polymers observed in sample PnBA–COOH 13 260, and a small amount of monofunctional polymers in sample PnBA–2COOH 11 450, because an AIBN initiator was used in the RAFT polymerization (see Table 2). It also can be seen clearly in Fig. 2 that the peaks were broader for the high-molecular-mass samples, which is in agreement with reported observations by Philipsen et al. [28] and others (see [17] and references cited therein). The better separation capabilities for low-molecular-mass polymers do not only result from thermodynamics (see

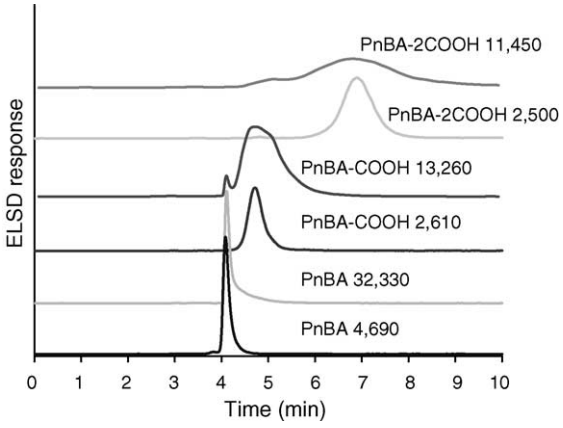


Fig. 2. Representative separations (chromatograms) of PnBA functional polymers according to COOH end-groups at 55 °C. Detection, ELSD; mobile phase, 5.7% ACN and 0.5% HAc in DCM; flow rate, 0.5 ml min^{-1}; injection volume, 10 μl; sample concentration, 1 mg ml^{-1} in DCM; column as in Fig. 1. For sample identification, see Table 2. *Note*: The peak heights of chromatograms were electronically adjusted for the sake of clarity.

Fig. 1d) but also from kinetics (peak broadening), as can be seen from Fig. 2. In this study we were dealing with low-molecular-mass PnBAs (around 1000–2500). These could easily be separated according to carboxyl functionality under the near-critical conditions of Fig. 2.

We selected three representative low-molecular-mass samples (PnBA 600, PnBA–COOH 2610, PnBA–2COOH 2500) and combined these into a single sample, which we then used to investigate the effects of mobile-phase composition and column temperature. Some of the resulting chromatograms are shown in Fig. 3. As shown in Fig. 3a, the base-line separation of this mixture of low-molecular-mass samples according to the carboxyl functionality was not affected when the column temperature was changed from 25 to 55 °C, although the retention times of mono- and difunctional polymers decreased somewhat when the temperature increased. However, it should be noted that this result may not be extrapolated to high-molecular-mass PnBAs (see Section 3.3). The effect of the mobile-phase composition is illustrated in Fig. 3b. Overlapping peaks were obtained when a mixture

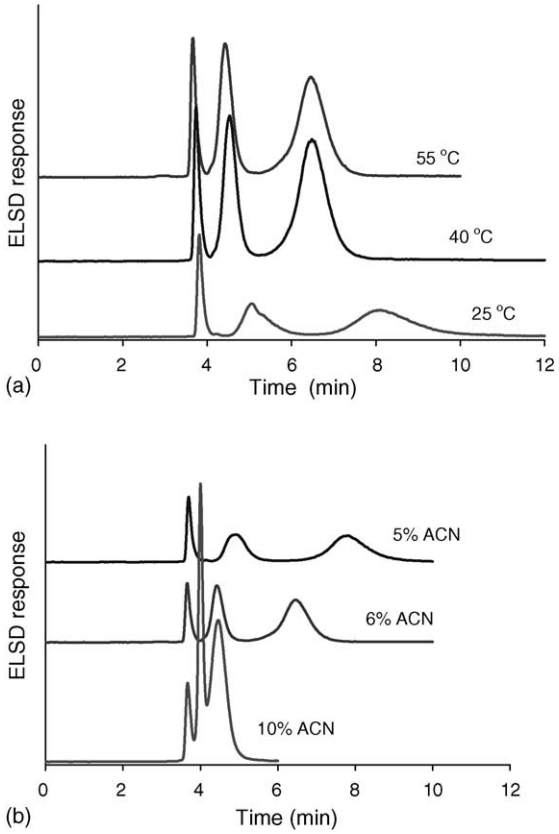


Fig. 3. Effect of temperature (a) and mobile-phase composition (b) on the separation of a mixture of PnBA with non-, mono-, and dicarboxyl functional polymers (PnBA 600, PnBA–COOH 2610 and PnBA–2COOH 2500). Detection, ELSD; flow rate, 0.5 ml min^{-1}; injection volume, 10 μl; sample concentration, 0.3 mg ml^{-1} (PnBA 600), 0.6 mg ml^{-1} (PnBA–COOH 2610) and 1.2 mg ml^{-1} (PnBA–2COOH 2500) in DCM; column as in Fig. 1. Mobile phase: (a) 6% ACN and 0.5% HAc in DCM; (b) varying percentage of ACN as indicated plus 0.5% HAc in DCM at 55 °C. *Note*: The peak heights of chromatograms were electronically adjusted for the sake of clarity.

of 10% ACN and 0.5% HAc in dichloromethane was used as the mobile phase. The separation of the three peaks was somewhat improved with 8% ACN (and 0.5% HAc) in DCM (not shown). Base-line separations of this mixture according to the carboxyl functionality were achieved when the mobile-phase composition was between 5 and 6% of ACN (and 0.5% HAc) in DCM at 55 °C.

The experimental results concerning near-critical solvent compositions for the PnBA samples at different HAc concentrations and different column temperatures are summarized in Table 3. A typical error for the precision in the estimated critical composition is ±0.3% (see Fig. 1a and c). In some cases, the confidence interval may be a bit wider (up to ±0.6%, see Fig. 1b). This implies that differences in the ACN (or DCM) concentrations of 0.5% in Table 3 are likely to be significant, whereas differences exceeding 1% are almost certainly significant. It can be seen in Table 3 that at increased acetic acid concentrations in the mobile phase lower ACN concentrations were required. This seems quite logical, because both HAc and ACN are polar solvents with higher elution strength than DCM on a silica column. Also, the near-critical solvent composition shifted to lower concentrations of ACN with increasing column temperature, because the interaction of the PnBA backbone with the silica column decreased. For example, the approximate critical composition for di-COOH-functional PnBA samples was 6.7% ACN and 0.5% HAc in DCM at 25 °C, while at 55 °C it was 5.5% ACN and 0.5% HAc. A mobile-phase composition of about 6.0% ACN and 0.5% HAc in DCM at 55 °C resulted in a slight decrease in retention with increasing molecular mass for di-COOH-functional PnBA samples. The same mobile phase at 25 °C showed common adsorption behavior, i.e. retention increased with increasing molecular mass [15]. As discussed further, higher temperatures can be used to completely elute high-molecular-mass PnBA samples at the near-critical composition (about 6.0% ACN and 0.5% HAc in DCM).

Formic acid was also tested instead of HAc as the modifier in the mobile phase. Because of the low boiling point of formic acid, it might be more favorable than acetic acid when coupling LC with MS. Near-critical solvent compositions for the PnBA samples, using 0.5% formic acid at column temperatures of 25 and 50 °C, are summarized in Table 4. The effect of the formic acid on the re-

Table 4
Approximate critical ("near-critical") compositions for PnBA samples obtained with a mobile phase containing 0.5% formic acid and at different column temperatures

Functionalities	Temperature (°C)	ACN (%)	DCM (%)
Non-COOH	25	10.2	89.3
	50	9.5	90.0
Mono-COOH	25	8.3	91.2
	50	7.5	92.0
Di-COOH	25	8.2	91.3
	50	7.4	92.1

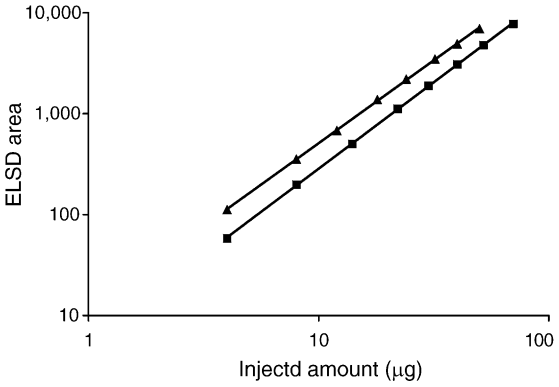


Fig. 4. ELSD calibration curves (logarithmic scale) for PnBAs with one and two carboxyl end-groups: (▲) PnBA–COOH 2610; (■) PnBA–2COOH 2500. Mobile phase, 6% ACN and 1.0% HAc in DCM; flow rate, 0.5 ml min^{-1}; column as in Fig. 1.

tention of carboxyl-functional PnBAs was similar to that of HAc.

3.2. Quantitative aspects

In order to obtain quantitative information on the FTD of the PnBA samples, as described in [22], ELSD had to be used. Because the ELSD response does not usually increase linearly with the polymer concentration, the calibration curves should be established carefully. An exponential calibration curve, such as in Eq. (1), is often used:

$$A = am_i^b \quad (1)$$

where A is the observed peak area, m_i is the injected sample amount (in mass units), and a and b are constants. The values of a and b can easily be determined from a logarithmic plot. As shown in Fig. 4, the ELSD calibration curves for mono- and di-COOH-functional PBAs established using samples PnBA–COOH 2610 (VL131) and PnBA–2COOH 2500 (VL127), respectively, can be described very well by Eq. (1). The values of a and b are shown in Table 5. We did not obtain a similar calibration curve for non-functional PBA, because no sufficiently pure standard was available. It can be seen from Fig. 4 and Table 5 that the COOH end-group had a significant influence on the ELSD response. This is due to the peak broadening encountered in interactive LC, which is greater for difunctional than for monofunctional polymers (see [29] for a more detailed explanation).

The calibration curves of Fig. 4 were used in the quantitative analysis of COOH-functional PBA samples under the specified LC conditions (6% ACN, 1.0% HAc in DCM, with a flow rate of 0.5 ml min^{-1} at 25 °C) assuming a negligible effect of molecular mass on the ELSD response in the low-molecular-mass range investigated [22]. Some representative results for functional polymers are shown in Table 6. It can be seen clearly in Table 6 that all the PnBA samples obtained from RAFT polymerization contained predominantly molecules with two carboxyl end-groups, as expected. The relative amount of monocarboxyl polymer chains was higher in the case of polymer VL103 than for VL068. This is consistent with the relatively high amount of AIBN initiator used in preparing VL103 (see Table 1), which is more than twice that used for preparing VL068 [6]. Higher percentages of dicarboxyl polymer chains in samples VL123 or VL127, as compared with sample VL103, can also be observed in Table 6. This result is expected from polymer chemistry. The ACVA initiator, which contains two COOH groups, was used for preparing both VL123 and VL127 (see Table 1). The initiator moiety, [HOOC$(CH_2)_2$C(CH_3)CN–] from ACVA, was introduced as one end-groups in some of the obtained RAFT polymers. The AIBN initiator, which contains no COOH groups, was used in case of VL103 [6]. The initiator moiety, [$(CH_3)_2$CCN–] from AIBN, was introduced as one end-group in some polymer chains (the other end-group containing the leaving group [HOOC$(CH_3)_2$C–] of the RAFT agent). This resulted in a relatively high percentage of monocarboxyl polymers in these samples. All these structures were confirmed by mass spectra (see discussion in Section 3.4). To achieve high carboxyl functionalities, either a COOH-containing initiator (ACVA) should be used, or a low concentration of a non-COOH-containing initiator (AIBN) relative to that of RAFT agent. In the latter case, the polymerization rate is much lower, which is not favorable in case of industrial application. Nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved for telechelic PnBAs prepared by one-step RAFT polymerization of PnBA.

Table 5
End-group effect on ELSD calibration curves for functional PnBAs

Sample	a	b	R^2
PnBA–COOH 2610	11.64	1.64	0.9998
PnBA–2COOH 2500	5.53	1.71	0.9999

LC conditions as in Fig. 4.

Table 6
Quantitative analysis of carboxyl-functional PnBAs by LC-ELSD

Sample	ELSD	
	Monofunctional (%)	Difunctional (%)
VL103 (PnBA–2COOH 2400)	3	97
VL123 (PnBA–2COOH 3200)	1	99
VL068 (PnBA–2COOH 2510)	1	99
VL127 (PnBA–2COOH 2500)	1	99

Isocratic conditions and column as in Fig. 4 (samples as indicated in Table 1).

3.3. Analyte recovery in isocratic LC of polymers

Although critical LC has been successfully employed for the separation of polymer blends [13,30], block copolymers [15,31] and for the separation of polymers based on functional groups or end-groups [18–22], there remains some controversy over whether precise co-elution conditions can indeed be achieved [28,32–34]. Also, reduced recovery has

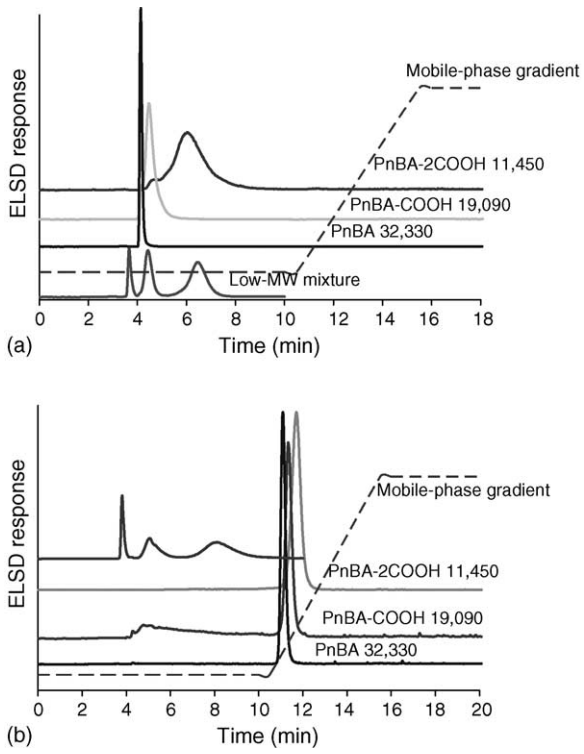


Fig. 5. Analyte recovery in isocratic LC of polymers. Column temperature: (a) 55 °C, all samples eluted at isocratic conditions; (b) 25 °C, high-molecular-mass samples eluted under gradient conditions, low-molecular-mass samples (top trace) eluted under isocratic condition. Mobile phase: first isocratic, 0–5 min, 6% ACN and 0.5% HAc in DCM; then 5–10 min, linear gradient to 15% ACN and 1.0% HAc in DCM (the dashed line indicates the composition of the mobile phase at the outlet of the column). Detection, ELSD; flow rate, 0.5 ml min^{-1}; injection volume, 10 μl; low-molecular-mass sample mixture, 0.3 mg ml^{-1} (PnBA 600), 0.6 mg ml^{-1} (PnBA–COOH 2610) and 1.2 mg ml^{-1} (PnBA–2COOH 2500) in DCM; high-molecular-mass samples, 2 mg ml^{-1} (PnBA 32 330, PnBA–COOH 19 090, PnBA–2COOH 11 450); column as in Fig. 1. See explanation in text. *Note*: The peak heights of chromatograms were electronically adjusted for the sake of clarity.

been observed for high-molecular-mass polymers at critical (or near-critical) conditions [35,36]. It is generally recommended to verify complete recovery in LC experiments on polymers to avoid misinterpretation. Following an approach described by Mengerink et al. [20], an isocratic mobile phase (near-critical conditions) was first employed during a time exceeding the sum of the dwell time and dead time. Then, the elution strength of the mobile phase was increased by programming a gradient to reach genuine exclusion conditions, where all the PnBA samples (either with or without COOH groups) were rapidly eluted. If we only observed peaks before the start of the gradient, no recovery problem was diagnosed, as in the case of Fig. 5a. All the PnBA samples were fully eluted under the near-critical (isocratic) conditions at 55 °C with 6% ACN and 0.5% HAc in DCM as the mobile phase. The retention time of the non-functional low-molecular-mass PnBA was lower than that of non-functional high-molecular-mass PnBA, but the retention time of the difunctional low-molecular-mass PnBA was slightly higher than that of difunctional high-molecular-mass PnBA as shown in Fig. 5a. With the same mobile phase at a different temperature of 25 °C, we observed peaks after the onset of the gradient for high-molecular-mass PnBA samples, as shown in Fig. 5b. We identified this as a recovery problem, because a fraction of the high-molecular-mass sample was not eluted at the initial isocratic critical conditions (during the time allowed). However, it is important to note that at the same conditions as in Fig. 5b, there were no recovery problems for low-molecular-mass PnBA samples (molecular masses up to 7000). The mono-hydroxyl RAFT-PnBA samples (VL28: M_p = 12 560, M_n = 8960, PDI = 1.32; VL29: M_p = 5430, M_n = 3980, PDI = 1.19) were also investigated using 6% ACN and 1.0% HAc in DCM at 25 °C. In this case, the recovery of the analyte was incomplete (not shown). This indicated that the interaction of OH end-groups with the stationary phase was not weaker than that of COOH end-groups under these conditions.

3.4. Mass spectrometric characterization

The near-critical solvent composition was applied to analyze the carboxyl-functional PnBA polymers obtained by RAFT polymerization. Some representative quantitative results for functional RAFT polymers are shown in Table 6. To confirm the critical separation of PnBA polymers based on the carboxyl functionality, MS was used. It is relatively straightforward to collect fractions and to perform off-line MS. Our aim was to synthesize telechelic PnBA with two COOH end-groups in one step. We did not observe a signal from the ELSD system at the position of the non-functional polymer. Therefore, two fractions were collected for each sample, at the positions corresponding to mono- and difunctional polymers (4.5–5.0 and 5.5–7.5 min, respectively).

The ESI-MS spectra for the two fractions of sample VL103 are shown in Fig. 6a and b. In the spectrum of Fig. 6b, a family of triplets is observed, each "triplet" being separated from the next by a mass of 128.1 u, which clearly corresponds to a single butyl acrylate (BA) monomeric unit. Within the triplets, the peaks are separated by 22 u. After subtracting the mass of the PnBA chain, the remainder of the main series of peaks (highest peaks of the triplets, one sodium cation) corresponded to the same structure as the RAFT agent used, which contained two leaving groups [$HOOC(CH_3)_2C-$] of the RAFT agent at the polymer-chain ends and the trithiocarbonate moiety inside the polymer chain. This structure is shown in Fig. 7a. It was further confirmed by ESI-CID-MS–MS. The weak C–S bonds between the acrylate chain and the thiocarbonate group easily break, releasing the trithiocarbonate moiety. The leaving group [$HOOC(CH_3)_2C-$] stays attached to the acrylate chain. A second sodium ion may exchange with the hydrogen ion of the carboxylic acid group. This does not affect the total charge of the ion, but it results in an increase in the observed mass of the polymer chain by 22 u. The polymer chains in VL103-2 contain two COOH groups. The substitution of zero, one or two hydrogens of the carboxylic acid groups explain the presence of a

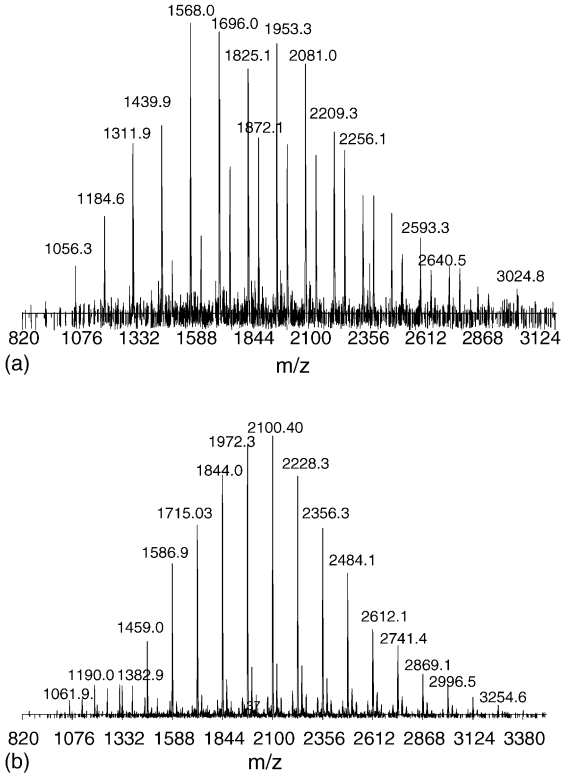


Fig. 6. ESI mass spectra for: (a) monofunctional fraction VL103-1 collected at 4.5–5.0 min; (b) difunctional fraction VL103-2 collected at 5.5–7.5 min. LC conditions: mobile phase, 6% ACN and 0.5% HAc in DCM; flow rate, 0.5 ml min^{-1} at 25 °C; column as in Fig. 1.

family of three peaks separated by 22 mass units. Also one minor series of peaks can be observed in the region of low mass-to-charge ratios (towards the left) in Fig. 6b. The main peaks in this series are separated by 64 u. This can be readily attributed to doubly charged ions with the same structure. Therefore, the structure of the VL103-2 fraction was confirmed to be poly(butyl acrylate) with dicarboxyl end-groups as shown in Fig. 7a.

In the spectra of the VL103-1 fraction in Fig. 6a, two different series can be observed with a repeating unit of 128.1 u, corresponding to a single BA monomeric unit (and singly charged ions). The remainder mass calculated for the main series of peaks (one sodium cation) is consistent with the leaving group [$HOOC(CH_3)_2C-$] of the RAFT agent and of the initiator AIBN moiety [$(CH_3)_2CCN-$] as the end-groups and a trithiocarbonate moiety inside the polymer chain. This structure is shown in Fig. 7b. It has also been confirmed by ESI-CID-MS–MS. This series was absent in the spectrum of the VL127-1 fraction (not shown), as is expected from polymer chemistry (see discussion in Section 3.2). However, we were not sure to assign another series of peaks with mass increments of 128.1 and shifted by +47 u relative to the main series. The ESI-CID-MS–MS spectrum indicated that this structure contained no weak bonds (C–S or other) in the middle of the polymer chain. This series was also present in the spectrum of the VL127-1 fraction. One possibility is that it is a product of chain transfer caused by an impurity in the RAFT agent. In any case, we conclude that the VL103-1 fraction contained poly(butyl acrylate) with one carboxyl end-group.

In a previous paper [8], we reported that the active (weak) bonds, such as dithioester moiety, in the RAFT PMMA samples led to easy fragmentation in MALDI-MS experiments. Nonaka et al. [37] suggested that the terminal C–Cl group of poly(methyl acrylate) (PMA) obtained by ATRP was relatively stable in comparison with that of PMMA during MALDI-TOF-MS analysis. Matyjaszewski et al. [38] reported that only very little fragmentation was observed in the MALDI-TOF-MS spectrum of PnBA synthesized by ATRP. Similarly, we observed less fragmentation in the MALDI-TOF-MS spectra for the RAFT-PnBA samples than for RAFT-PMMA. When the experimental conditions were carefully tuned (reducing the laser energy), intact molecular ions were observed in the MALDI-TOF-MS spectra. An example is shown in Fig. 8, which represents the MALDI-TOF-

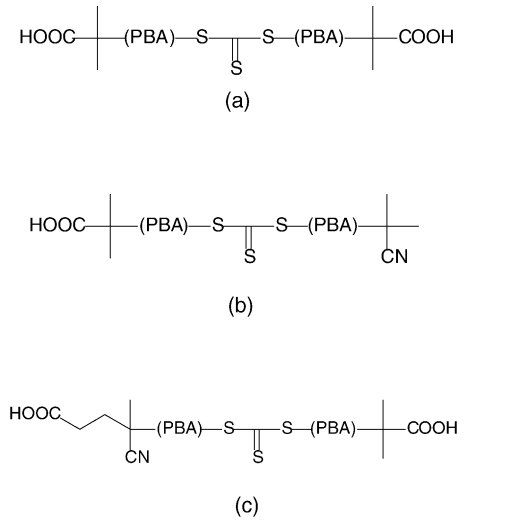


Fig. 7. The main structures of carboxyl-functional PnBA samples synthesized by RAFT polymerization.

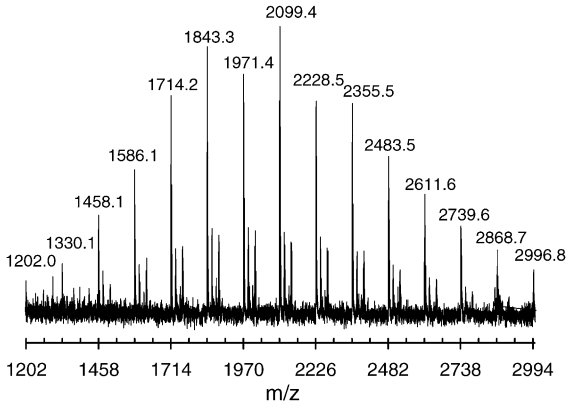


Fig. 8. MALDI-TOF mass spectrum for difunctional fraction VL127-2 collected at 5.5–7.5 min. LC conditions as in Fig. 6.

MS spectrum of the difunctional fraction VL127-2. Three different series can be seen in Fig. 8 with repeating units of 128.1 u, again corresponding to the BA monomeric unit. The main series of peaks results from the difunctional PnBA polymers with one sodium cation (Na^+). This series contains two leaving groups [$HOOC(CH_3)_2C–$] of the RAFT agent at the polymer-chain ends. This is the same structure as that of sample VL103-2 (see Fig. 7a). The first minor series can be assigned to the potassium cation (K^+) series (with mass increments of 16 u relative to the Na^+ series). The second minor series arises from another type of difunctional PnBA polymers with one sodium cation (Na^+), which contains one end-group [$HOOC(CH_3)_2C–$] from the RAFT agent and one end-group [$HOOC(CH_2)_2C(CH_3)CN–$] from the initiator moiety. This structure, which is expected from polymer chemistry, is shown in Fig. 7c. Therefore, only dicarboxyl-terminated structures were identified in this fraction of VL127-2.

It should be mentioned here that the monofunctional polymers identified in samples VL127 and VL068 could not be clearly observed by ESI-MS or MALDI-TOF-MS without prior LC separation of the sample. Therefore, critical LC coupled with MS provides additional information about the polymer structure.

4. Conclusions

The (near-) critical solvent compositions for non-, mono-, and difunctional carboxyl-PnBAs were determined in normal-phase LC, using mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying composition and a bare-silica column. Some formic or acetic acid had to be added to the mobile phase to elute PnBA polymers with carboxyl end-groups. The critical solvent compositions obtained were not identical for non-, mono-, and difunctional PnBA–2COOH samples. Because both acetic acid and acetonitrile are polar solvents with higher elution strengths than DCM on a silica column, lower acetonitrile concentrations were required when increasing the acetic acid concentration in the mobile phase. Formic acid behaved similar to acetic acid. Low-molecular-mass PnBA samples were successfully separated according to carboxyl functionality at (near-) critical conditions (6% ACN and 0.5% HAc in DCM at 55 °C or 6% ACN and 1.0% HAc in DCM at 25 °C). Isocratic, near-critical LC of high-molecular-mass PnBAs proved feasible, but this required elevated temperatures (55 °C).

Under appropriate conditions, reliable ELSD calibration curves could be obtained and these were used for the quantitative analysis of carboxyl-functional RAFT-PnBA prepolymers. The results from LC at the near-critical conditions showed that all the obtained PnBA samples based on RAFT polymerization mainly contained (telechelic) molecules with two carboxyl end-groups. Mass spectra confirmed that the critical separation of PnBA polymers was based on the carboxyl functionality. Critical LC coupled with MS provided a great deal of information on the polymer structure. The quantitative data and MS spectra were consistent with the expected results from the mechanism of the RAFT polymerization. To achieve high carboxyl functionalities, either a COOH-containing initiator (ACVA) or a very low concentration of a non-COOH-containing initiator (AIBN) should be used. In the latter case the polymerization rate is much lower. Near-ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved for telechelic PnBAs prepared by one-step RAFT polymerization of PnBA.

Acknowledgements

This project is funded by the Dutch Polymer Institute (DPI project 205). We thank Aschwin van der Horst (University of Amsterdam), Rajan Venkatesh and Professor Rob van der Linde (Eindhoven University of Technology) for their cooperation. Helpful discussions with Professors A.M. Skvortsov and A.A. Gorbunov from St. Petersburg (Russia) were made possible by INTAS (project No. INTAS-OPEN-2000-0031).

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