e-Polymers **2005**, no. 042.

http://www.e-polymers.org
ISSN 1618-7229

High-throughput screening of the chemical composition distribution of random styrene-butyl acrylate copolymers

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(Received: March 3, 2005; published: June 23, 2005)

Abstract: The development of high-throughput liquid chromatographic techniques for the analysis of styrene-butyl acrylate (SBA) copolymers is discussed. The analysis time in size-exclusion chromatography (SEC) can be reduced to about 3 min per sample when high-throughput SEC columns and high flow rates are used. In gradient HPLC, small columns with improved separation efficiencies can be applied. The time requirements can be decreased to less than 2 min per sample. Using the high-throughput HPLC technique, the chemical composition distribution of high-conversion SBA copolymers can be analyzed in a fast and efficient way. The calibration of HPLC separation is conducted by coupling the HPLC system with FTIR through the LC-transform interface. A comparison of the chemical compositions of the copolymers obtained by ^{1}H NMR, off-line FTIR and coupled HPLC-FTIR verifies the accuracy of the high-throughput copolymer analysis approach.

Introduction

Combinatorial methods are high-efficiency methods to create large composition libraries of materials, e.g., continuous composition variations. These compositions are tested systematically in parallel for specific properties of interest, in contrast to the time-consuming one-composition-at-a-time approach. Combinatorial methods are quite advanced in the pharmaceutical industries, but less developed in materials research. However, in recent years combinatorial methods have captured the attention of materials industry with the promise of providing new discoveries “faster, better, and cheaper” [1-3].

Combinatorial methods are of particular interest for the development of new or better polymeric materials. Given the huge variety in monomers, catalysts, and polymerisation techniques leading to the possible preparation of unlimited numbers of new formulations it is reasonable, if not obvious, to use combinatorial techniques. The ability to map out structure-property relationships in a relatively short period of time is crucial for the discovery of polymers with new or improved properties [4-6].

The preparation of large numbers of target compounds is only the first step in a combinatorial set-up. As important as the fast synthesis is the fast analysis of the prepared sample sets. This, however, is much more complicated for polymers as compared to drug molecules or other low-molar-mass species. Synthetic polymers are highly complex materials that very frequently exhibit a chemical or functional heterogeneity in addition to the molar mass distribution. In order to evaluate a new material properly, the different distributions have to be determined quantitatively [4].

Liquid chromatographic techniques are best suited for the analysis of the different polymer distributions. SEC is the established method for the analysis of molar mass distributions, while interaction chromatography (e.g., HPLC or LC-CC) is used to analyse the chemical heterogeneity or the functionality type distribution [4]. Using conventional set-ups, the analysis of one sample by SEC or HPLC takes not less than about 20 - 30 min.

It has been shown in previous years that the throughput in SEC can be increased by 5 - 10 times when new stationary phases and column shapes are developed [7-9]. Using these new materials the time per SEC run can be decreased to about 2 min. When new column technology is applied to HPLC of polymers, functionality type separations can be conducted in less than 5 min [9-10]. However, while the chromatographic separation can be conducted in a very short period of time, the spectroscopic (e.g., FTIR) analysis of chromatographic fractions takes much longer.

The present article discusses the high-throughput analysis of random styrene-acrylate copolymers with regard to molar mass and chemical composition distribution. The molar mass analysis shall be conducted using high-resolution fast SEC columns. For the analysis of the chemical composition distribution, the copolymers shall be separated by gradient HPLC and analysed by FTIR spectroscopy. The coupling of the two techniques shall be carried out through the LC transform interface to enable the fast and reliable FTIR analysis of the HPLC fractions.

Results and discussion

Copolymer synthesis

Low- and high-conversion random copolymers of styrene and butyl acrylate were prepared in a parallel experimental set-up. 13 parallel polymerisations were carried out in a Polymer Laboratories PL-SP 260 high-temperature sample preparation instrument. Monomers, initiator and solvent were placed into screw-neck vials of 4 mL. The vials were heated to a temperature of 70°C and agitated. After a polymerization time of 1 h for low-conversion and 20 h for high conversion, the polymer solutions were precipitated in methanol and dried in vacuum. Monomer ratios, solvent, and initiator concentrations are summarized in Tab. 1: low-conversion copolymers see samples 1 - 6, high-conversion copolymers see samples 8 - 14. Poly(butyl acrylate) (PBA, sample 7) was a technical sample purchased from Röhm GmbH, Darmstadt, Germany.

Molar mass analysis of the copolymers by fast SEC

As was described in the Introduction, fast SEC columns for high-throughput analysis of polymers have been developed in recent years. In particular, high-speed SEC columns with good resolution have been introduced recently by Polymer Standards Service GmbH, Mainz, Germany. Using these columns SEC separations can be conducted within about 2 min. The two important features of the high-speed PSS columns are (1) a new packing material that overcomes the pore access limitations of conventional columns by introducing channel-type pores, and (2) a change in the column aspect ratio. The optimum column dimensions have been found to be 50 mm in length and 20 mm internal diameter.

Tab. 1. Monomer ratios, solvent, and initiator concentrations of the reaction mixtures; BPO: benzoyl peroxide

Sample	Butyl acrylate content in mol-% / g	Styrene content in mol-% / g	BPO conc. in 10^{-2} mol/L	Toluene vol. in µL
1	0/0	100/0.9	2.5	-
2	20/0.23	80/0.71	2.5	-
3	30/0.33	70/0.62	2.5	-
4	50/0.53	50/0.43	2.5	-
5	70/0.72	30/0.25	2.5	-
6	80/0.82	20/0.17	2.5	-
7	100	0	-	-
8	20/0.23	80/0.71	2.5	-
9	30/0.33	70/0.62	2.5	-
10	40/0.43	60/0.53	2.5	-
11	50/0.53	50/0.43	2.2	50
12	60/0.63	40/0.34	2.1	100
13	70/0.72	30/0.25	2.0	150
14	80/0.82	20/0.17	1.9	200

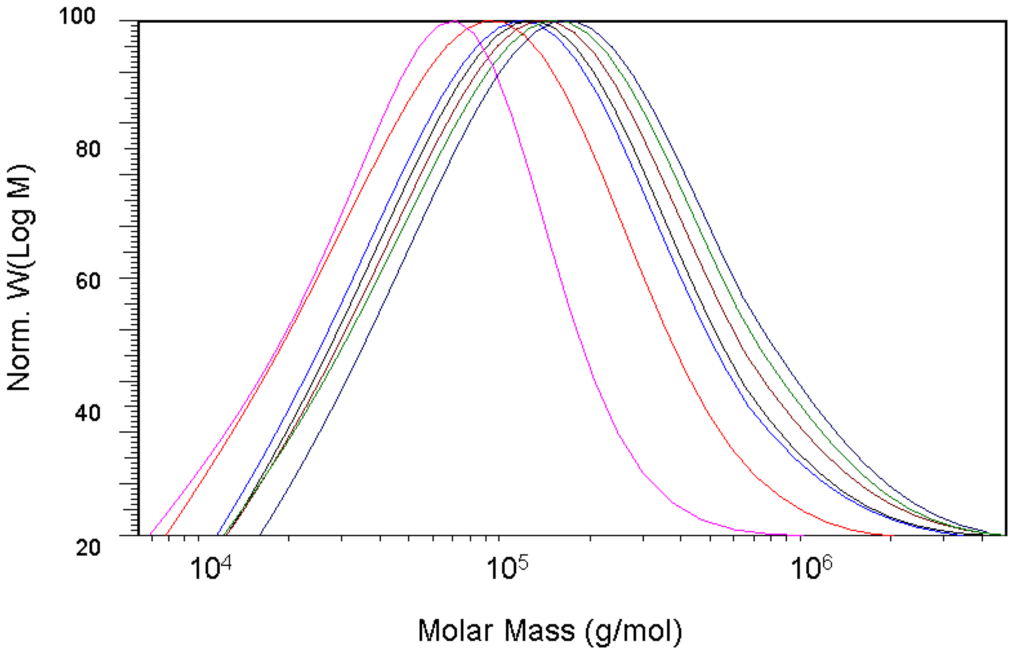

Fig. 1. Molar mass distributions of the low-conversion SBA copolymers; stationary phase: linear SDV XL 10µ; mobile phase: tetrahydrofuran (THF); detector: refractive index (RI); samples (from left to right): 7 (pink), 1 (red), 2 (blue), 3 (black), 4 (brown), 5 (green), 6 (dark blue)

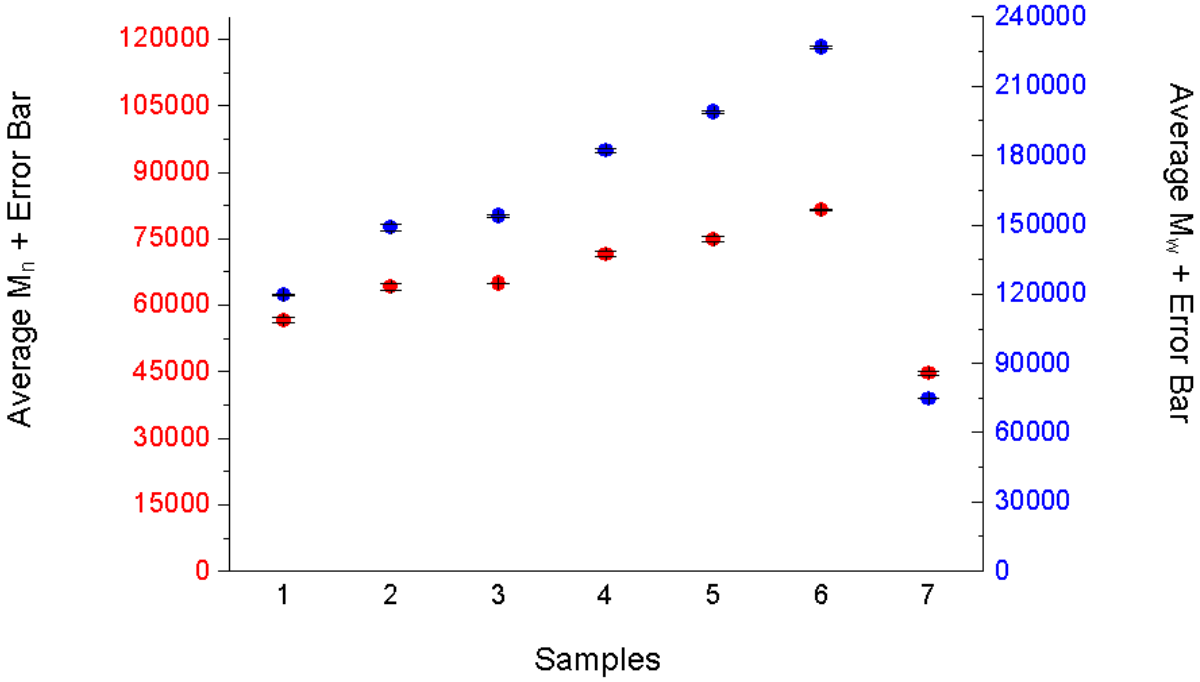

Fig. 2. Error plot for molar mass analyses of narrow-dispersed SBA copolymers; M_n (red) and M_w (blue)

A linear SDV (styrene-divinylbenzene) high-throughput column (separation range from 10^3 to $3x10^6$ g/mol) exhibited the best performance. Accordingly, this column was selected for the subsequent SEC analyses of the copolymers. To separate a mixture of seven PS calibration standards with molar masses between 376 and 579 000 g/mol an elution volume of 16 mL was required. Using a flow rate of 5 mL/min the time requirement per analysis was 3 min. As compared to a conventional SEC column where the time requirement would have been roughly 20 min per analysis this corresponds to a time saving of more than 80%. Using these SEC conditions, the low-conversion SBA copolymers were analysed with regard to molar mass distribution, see Fig. 1.

For each copolymer five measurements were carried out in order to evaluate the experimental error in SEC. Using overlapping between injection and elution, 35 analyses were carried out within only 105 min. The error plot in Fig. 2 presents average molar mass values and error bars for each copolymer. Typical experimental errors in the present experiments were around 1%.

Under the same high-throughput SEC conditions the high-conversion samples were analysed, see Fig. 3. Here again, it can be assumed that the experimental error is very low. The molar masses of all samples are summarized in Tab. 2.

Fast gradient HPLC of the copolymers

Random styrene-n-butyl acrylate copolymers are typically complex mixtures, which are distributed in molar mass and chemical composition. Depending on the different reactivities of styrene and n-butyl acrylate in the copolymerization reaction, the instantaneous composition of the copolymer molecules differs from the composition of the monomer mixture. Depletion of styrene in the monomer mixture with increasing conversion causes subsequent portions of the copolymer to be polymerised from monomer mixtures of different compositions. Using appropriate chromatographic conditions, the resulting chemically heterogeneous copolymers can be separated with respect to their chemical composition distribution (CCD) in the order of increasing n-butyl acrylate content.

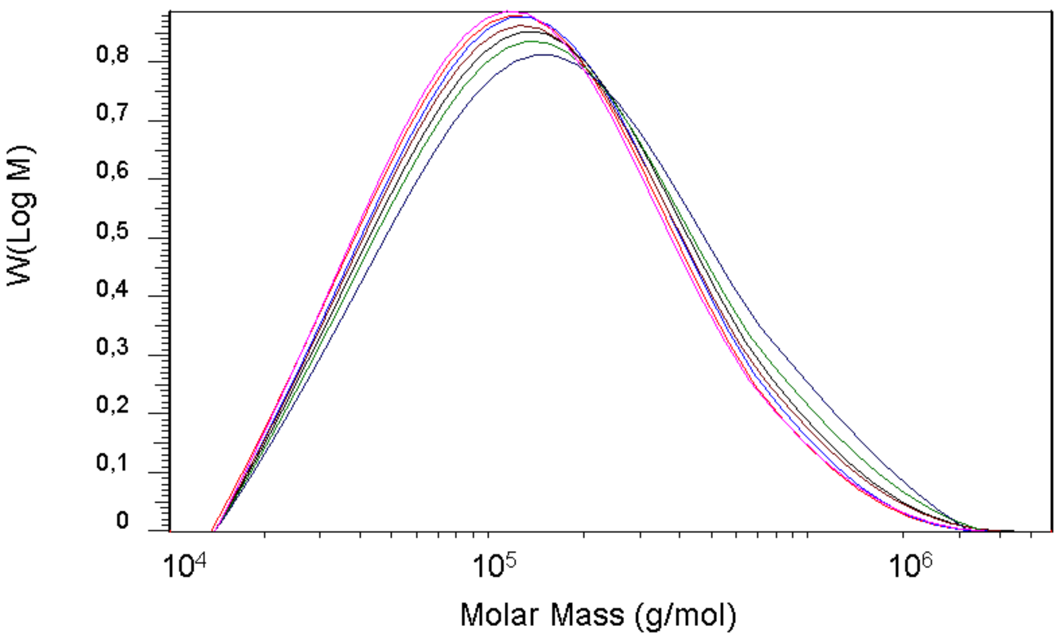

Fig. 3. Molar mass distributions of the high-conversion SBA copolymers; stationary phase: linear XL SDV 10µ; mobile phase: THF; detector: RI; samples (from left to right): 14 (pink), 8 (red), 9 (blue), 10 (black), 11 (brown), 12 (green), 13 (dark blue)

Tab. 2. Molar masses and relative standard deviations (RSD) of the SBA copolymers analysed by high-throughput SEC with RI detection

Sample	M_w / (kg·mol^{-1})	RSD of M_w in %	M_n / (kg·mol^{-1})	RSD of M_n in %
1	120	0.2	57	1.0
2	149	0.9	64	1.0
3	154	0.2	65	0.0
4	182	0.7	72	0.5
5	199	0.2	75	0.6
6	227	0.2	82	0.1
7	75	0.4	44	1.0
8	223		88	
9	230		89	
10	256		93	
11	247		91	
12	276		96	
13	299		101	
14	223		86	

A very useful liquid chromatographic technique for copolymer separation is gradient HPLC. As has been shown by Glöckner and others, under favourable conditions gradient HPLC separates copolymers strictly with regard to chemical composition rather irrespective of molar mass [11]. For styrene-ethyl acrylate copolymers it was shown by Krämer et al. that they can be separated according to chemical composition using a RP-18 stationary phase and a linear gradient of acetonitrile-THF [12]. At a flow rate of 1 mL/min the time requirement per analysis was roughly 20 min.

For the analysis of styrene-butyl acrylate copolymers the chromatographic system proposed by Krämer was not suitable. In this case a gradient of hexane-toluene-2-butanone on a silica gel stationary phase was found to separate the copolymers according to chemical composition. As it was the aim of the present study to run fast HPLC separations, a very short column (Luna of Phenomenex) with an average particle size of 3 µm was used. Column size was 30 x 4.6 mm I.D. The gradient is given in Tab. 3.

Tab. 3. Gradient for the HPLC separation of SBA copolymers

Time in min	Hexane content in %	Toluene cont. in %	2-Butanone cont. in %
0	60	40	0
0.15	33	60	7
0.60	45	40	15
1.5	20	40	40
1.75	100	0	0
2.25	100	0	0

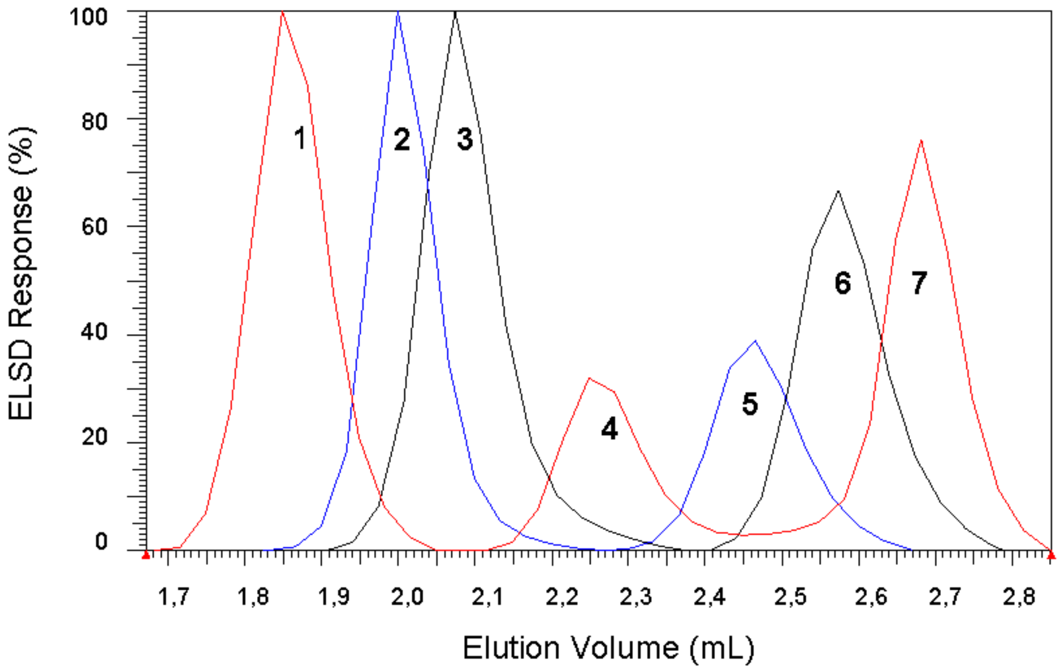

Fig. 4. Gradient HPLC of blends of PS, PBA and different low-conversion SBA copolymers; stationary phase: Luna of Phenomenex; eluent: hexane-toluene-2-butanone; detector: evaporative light scattering (ELS); blend 1 (red): samples 1+4+7, blend 2 (blue): samples 2+5, blend 3 (black): samples 3+6

Fig. 4 shows the chromatograms of the different low-conversion SBA copolymers. As can be seen, very narrow and uniform elution peaks are obtained. Since gradient HPLC separates according to chemical composition, the narrow and uniform elution peaks are a clear confirmation for the fact that these samples have a very narrow chemical composition distribution.

The separations were conducted at a flow rate of 2 mL/min and a column temperature of 40°C. At this flow rate the time requirement per analysis is less than 1.5 min. The total analysis time for the three blends including column washing and re-establishment of the gradient conditions took less than 8 min. Accordingly, this technique can be used for the fast separation of large numbers of samples with regard to chemical composition. As compared to conventional gradient HPLC separation experiments timesavings of more than 90% were achieved.

The multiple separation of blends of narrow distributed SBA copolymers is presented in Fig. 5. Reproducibility of the separation was analyzed by comparing the elution volumes of copolymer peaks at peak maximum. As can be seen in Fig. 5, the reproducibility of the method is excellent.

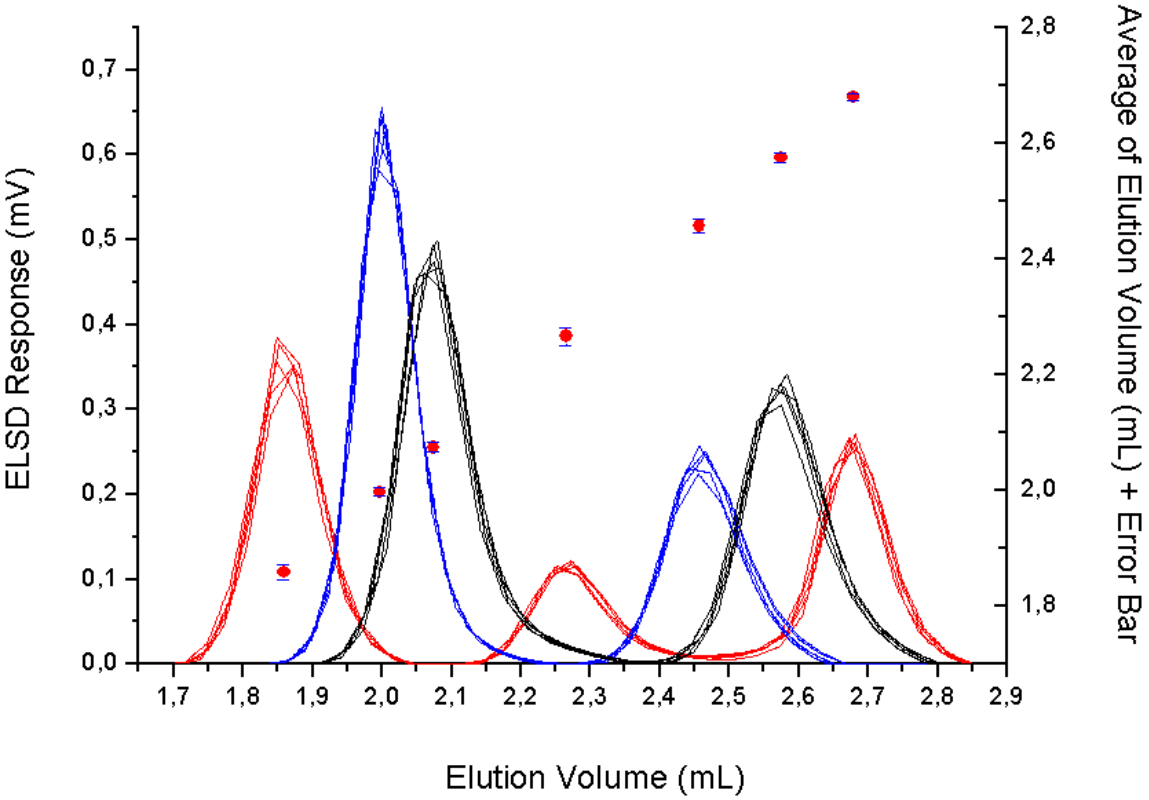

Fig. 5. Multiple HPLC separations of blends of SBA copolymers; experimental conditions and sample assignment see Fig. 4

Fast FTIR analysis of chromatographic fractions

For the analysis of copolymers with regard to chemical composition distribution, the HPLC system must be calibrated, i.e., a correlation of the elution volume and the chemical composition must be established. Using the calibration function, the actual chemical composition across a chromatographic peak can be determined in a fast and efficient way.

In the present case, the calibration was conducted by measuring blends of polystyrene and poly(butyl acrylate) in different well-known proportions. From the FTIR spectra of the blends the ratios of the absorption peaks at 700 cm^{-1} for polystyrene (I_{PS}) and 1727 cm^{-1} for poly(butyl acrylate) (I_{PBA}) were determined and plotted against the poly(butyl acrylate) content of the blend, see Fig. 6. The calibration curve was used subsequently to calculate the butyl acrylate contents of the copolymers from the FTIR peak areas.

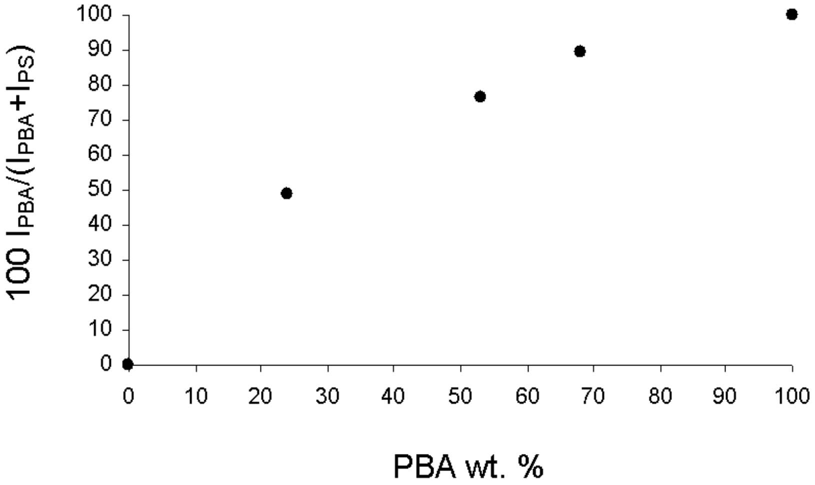

Fig. 6. Calibration curve for the analysis of SBA copolymers with regard to chemical composition

The fast compositional analysis of chromatographic peaks can be conducted using FTIR as a direct detector in HPLC. To hyphenate chromatography with FTIR a so-called LC-transform interface is used. The design concept and the operation of the interface are briefly described in refs. [14-16]. As the result of the measurements chemigrams are obtained presenting the intensity profile of a certain absorption band across the elution profile.

The deposited copolymer fractions that were eluted from HPLC and sprayed onto the Germanium disc are shown in Fig. 7. As can be seen the whole disc can be loaded with HPLC fractions that automatically are scanned in the FTIR spectrometer.

Fig. 7. Germanium disc loaded with SBA fractions from HPLC separation

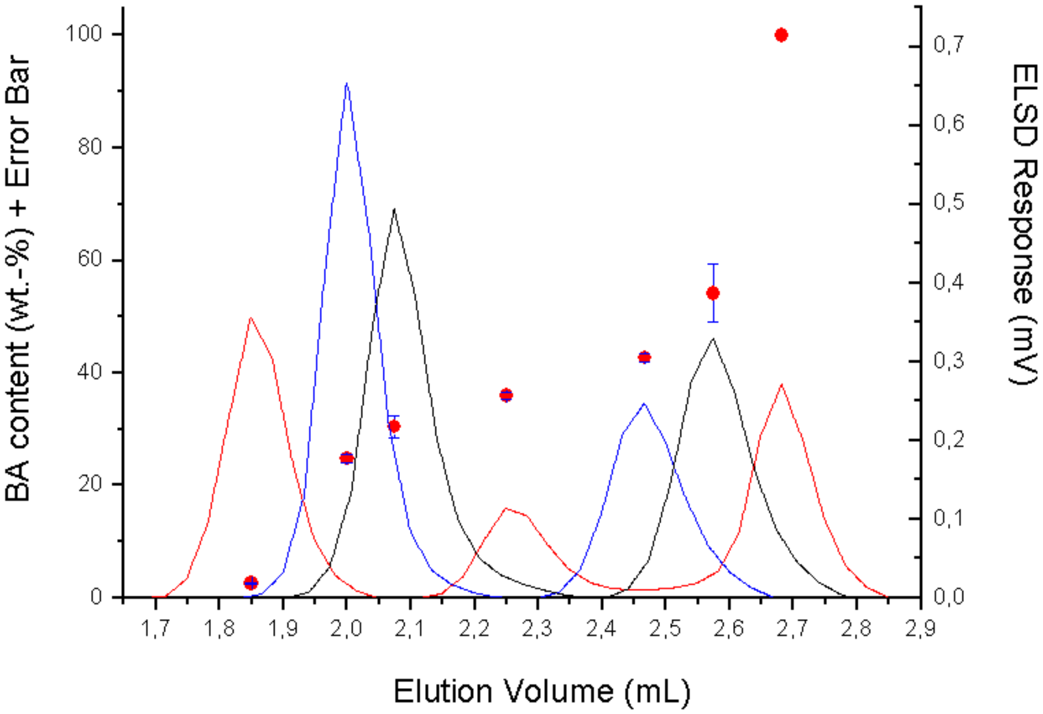

Fig. 8. Multiple HPLC separations of blends of SBA copolymers and analyses of the BA content by FTIR; experimental conditions and sample assignment see Fig. 4; errors are indicated by corresponding error bars

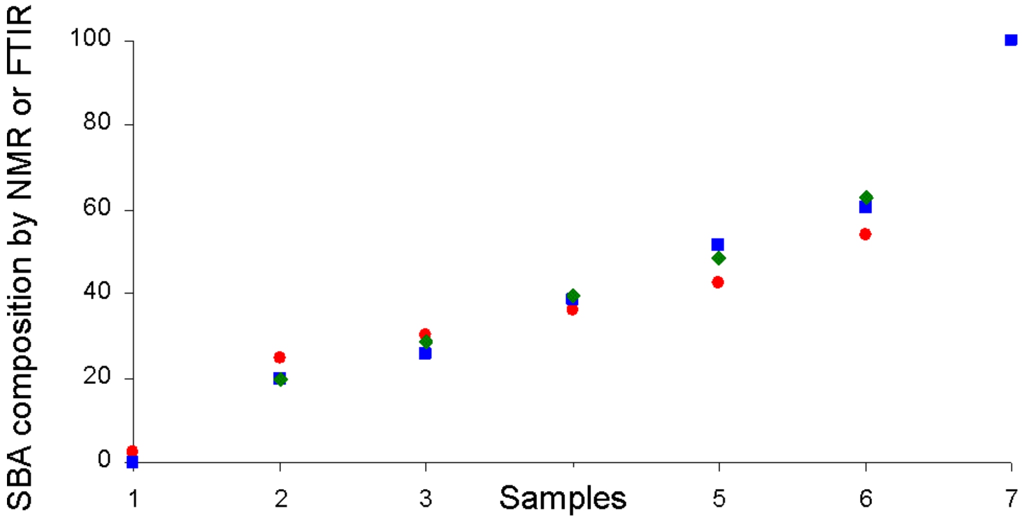

Fig. 9. Comparison of SBA chemical composition as determined by ^{1}H NMR (blue squares), off-line FTIR (green diamonds), and coupled HPLC-FTIR (red circles)

After optimization of the FTIR settings, the measurement of the background and of seven copolymer peaks can be carried out in less than 6 min. The low-conversion copolymers were measured within 18 min, while with conventional measurements this would have taken more than 3 h. Fig. 8 shows the HPLC-FTIR results of the copolymer blend separations. As has been shown in Fig. 4, one blend with three polymer components and two blends with two copolymer components were analyzed. The calculated butyl acrylate contents of the copolymer fractions are plotted as a function of the elution volume. To check for reproducibility, every copolymer blend was separated and analyzed consecutively three times. The accuracy of these measurements is presented as an error plot. Considering the errors in the calibration of FTIR, the evaluation of sample spectra and the experimental peculiarities of the LC-transform system, a maximum error of about 10% can be regarded as a good result.

To check the accuracy of the FTIR analyses, the data of ^{1}H NMR and FTIR bulk analyses were compared with the results of the coupling experiments. The ^{1}H NMR measurements were conducted in deuterated chloroform, for the determination of the copolymer compositions the signals of the aromatic protons (styrene) and the -CH_2O-protons (ethyl acrylate) were used. As can be seen in Fig. 9, a very good agreement between the different techniques was achieved. Maximum error was less than 9%.

For the analysis of the high-conversion SBA copolymers the same gradient conditions were used. Different from the low-conversion copolymers these copolymers exhibit very broad elution peaks, see Fig. 10. This is a clear indication of the high chemical heterogeneity of these copolymers.

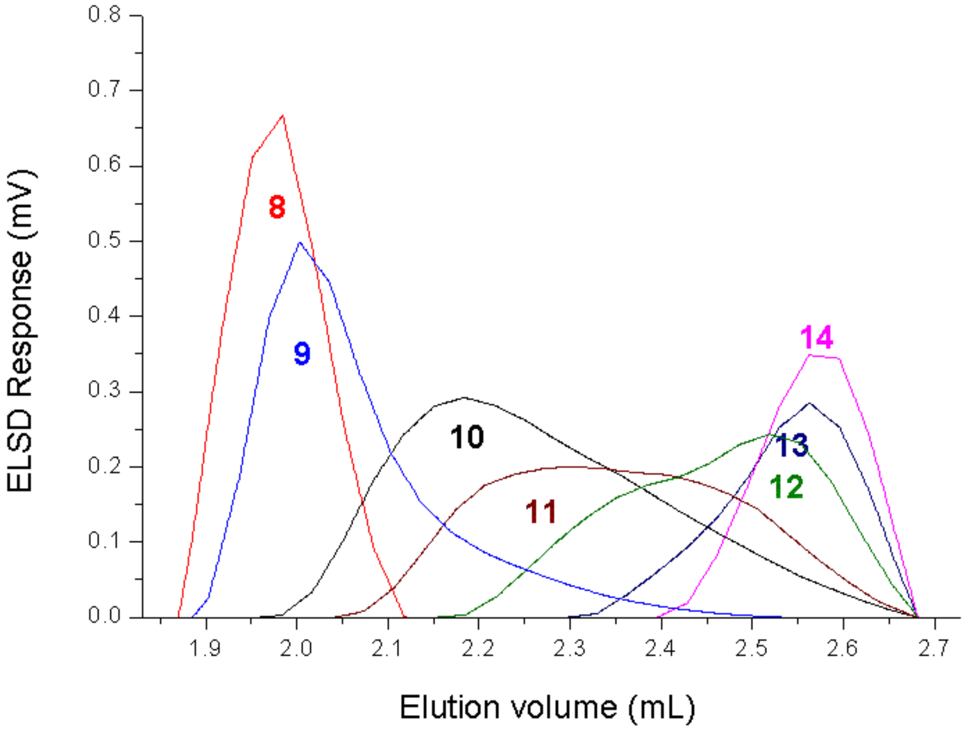

Fig. 10. Gradient HPLC analyses of high-conversion SBA copolymers; experimental conditions see Fig. 4. Numbers indicate sample numbers

Using the BA content vs. elution volume dependence given in Fig. 8, the elution curves of the high-conversion SBA copolymers in Fig. 10 can be re-calculated to chemical composition distribution curves, see Fig. 11.

Considering the limited accuracy of the concentration axis, these curves give a very clear representation of the chemical heterogeneity of the copolymers. Depending on the composition of the monomer feed, distribution curves of different shape and broadness are obtained. For example, the copolymer with a nominal content of 50% BA contains copolymer fractions with BA contents of 28 - 97%.

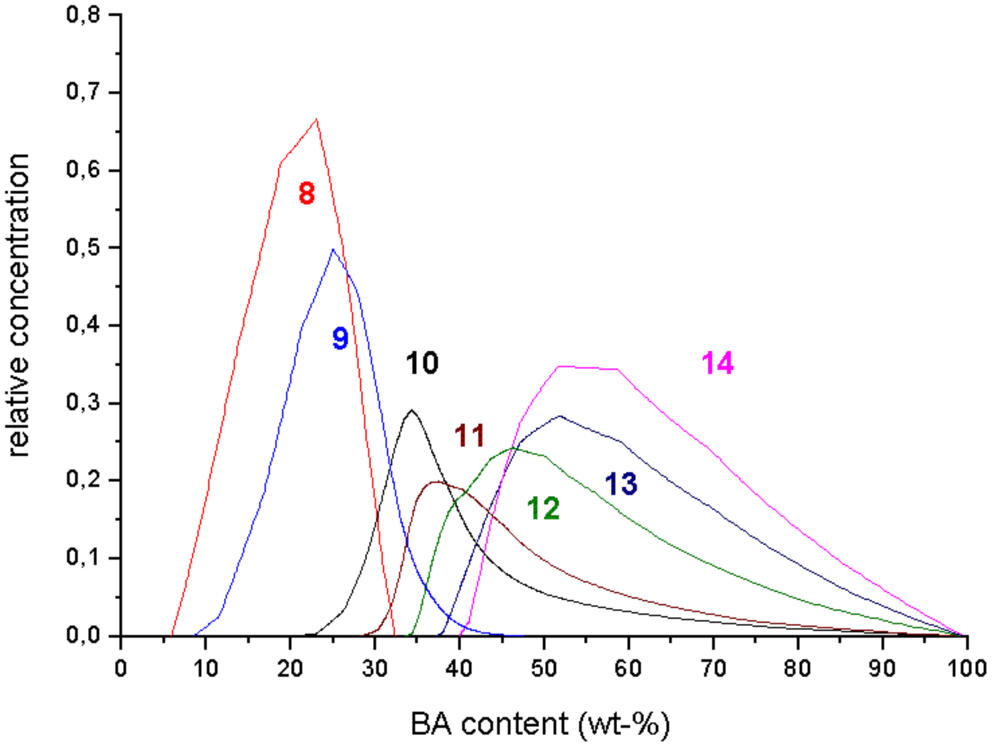

Fig. 11. Chemical composition distribution (CCD) of high-conversion SBA copolymers

Conclusion

The fast and reproducible chromatographic analysis of styrene-butyl acrylate copolymers can be achieved when existing techniques are modified with regard to the stationary and mobile phases, and the flow rate. High-throughput SEC analyses can be conducted within less than 3 min per sample working at a flow rate of 5 mL/min. The time requirements for gradient HPLC separations can be decreased to less than 2 min per sample when very short and highly efficient columns and high flow rates are used. Using the high-throughput HPLC technique, the chemical composition distribution of high-conversion SBA copolymers can be analyzed in a fast and efficient way. The calibration of the HPLC separation was conducted by coupling the HPLC system with FTIR through the LC-transform interface. A comparison of the chemical compositions of the copolymers obtained by ^{1}H NMR, off-line FTIR and coupled HPLC-FTIR verified the accuracy of the high-throughput copolymer analysis approach.

Experimental part

Polymers

Thirteen random copolymers were prepared using a parallel experimental set-up. Polymerisations were carried out in a Polymer Laboratories PL-SP 260 high-temperature sample preparation instrument. Screw-neck vials of 4 mL volume were filled with destabilized styrene and n-butyl acrylate, recrystallised benzoyl peroxide and distilled toluene. The vials were agitated and heated at a temperature of 70°C. For the low-conversion samples the polymerisation time was one hour, for the high-conversion samples the reaction time was twenty hours. In this case the amount of toluene was optimized in order to avoid cross-linking. The copolymer solutions were precipitated in methanol and dried in vacuum at 40°C. The copolymer blends were prepared by mixing solutions of the copolymers in toluene.

Chromatographic system

An Agilent 1100 Series HPLC modular system (Agilent Technologies) comprising a quadruple pump, an auto-sampler and a column oven was used. For data collection and processing the software package 'WinGPC-Software' (Polymer Standards Service, Mainz, Germany) was used.

Columns and mobile phases

SEC measurements were conducted on a high-speed column linear SDV of PSS GmbH, Mainz, Germany. The average particle size was 10 µm. Column size was 50 x 20 mm I.D. The SEC eluent was tetrahydrofuran. The samples were dissolved in a concentration of 0.5 mg/mL and filtered through a 0.45 µm filter prior to analysis.

HPLC measurements were carried out on a stationary phase Luna silica gel of Phenomenex. The average particle size was 3 µm. Column size was 30 x 4.6 mm I.D. A solvent gradient of hexane-toluene-2-butanone was used. The samples were dissolved in a concentration of 6 mg/mL in toluene for the HPLC experiments. All sample solutions were filtered through a 0.45 µm filter prior to analysis. A volume of 5 µL of the solution was injected for HPLC calibration and 20 µL for FTIR measurements on the disc.

Detectors

Polymer Laboratories evaporative light scattering detector ELS 1000, gas flow: 1.5, nebulizer temperature: 90°C, evaporation temperature: 120°C. Agilent 1100 Series refractive index detector, temperature: 35°C.

LC-FTIR Coupling

For these experiments the LC-transform interface LCT-1 was used. Experimental conditions were as follows: evaporation temperature 110°C, rotation of the Germanium disc with variable speed at 45°/min. The FTIR spectra were taken with a Nicolet Protégé^{tm} 460 Magna-IR Technology spectrometer, one sample and background scan, 8 cm^{-1} spectral resolution, 600 - 2000 cm^{-1} spectral range.

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