Full Paper: Random poly[(styrene)-*co*-(ethyl acrylate)]s can effectively be analysed with respect to the chemical composition distribution by on-line coupled HPLC/^{1}H NMR. The separation of the copolymers is conducted on a reversed-phase column using acetonitrile/tetrahydrofuran as the eluent mixture. Via on-line coupling, the chromatographic peaks are directly transferred into the NMR spectrometer and analysed on-flow. Information on the chemical composition and the chemical heterogeneity of copolymers with high conversion is obtained. The experiments have been carried out using conventional HPLC-grade solvents and no deuterium lock. The results of the on-line HPLC/^{1}H NMR investigation have been correlated with a HPLC procedure based on calibration with narrow-distributed copolymer standards.

On-line coupling of gradient-HPLC and ^{1}H NMR for the analysis of random poly[(styrene)-*co*-(ethyl acrylate)]s

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Introduction

Random copolymers are heterogeneous with respect to molar mass and chemical composition. The chemical composition distribution (CCD) is a result of the different reactivities of the monomers in the polymerization process, the different sequence of incorporation into the polymer chain, and the different chain termination reactions. The CCD can be determined efficiently by gradient high-performance liquid chromatography (HPLC). In a fundamental paper Teramachi et al. separated copolymers of styrene and methyl acrylate according to chemical composition using an adsorption-desorption mechanism and the gradient elution technique.[1] Since then, gradient elution has been applied to several types of copolymers; an exhaustive overview on different applications has been given by Glöckner several years ago.[2] It has been found that in many cases the molar mass effect is negligible when an adsorption mechanism is responsible for the separation.

Quantitative information on the chemical heterogeneity is obtained by calibrating the chromatographic system with chemically narrow-distributed low-conversion copolymers. By determining the retention time at the peak maximum for different samples of known composition, a calibration curve of retention time versus composition is constructed. This calibration curve is used for calculating the CCD. Therefore, the determination of the CCD of a particular sample necessitates the synthesis of a set of calibration samples which makes the whole procedure rather time consuming and laborious.

A much more feasible procedure would be the direct monitoring of the chemical composition across the elution curve. This is possible when the liquid chromatographic separation is directly coupled to a spectroscopic detection technique, preferably ^{1}H NMR. The combination of liquid chromatography with NMR has been attempted numerous times. Early experiments of coupled SEC/^{1}H NMR were conducted in a stop-flow mode or with very low flow rates.[3–5] This was necessary to accumulate a sufficient number of spectra per sample volume in order to improve the signal-to-noise ratio. For example, Hatada et al. described the analysis of isotactic polymethyl methacrylate by on-line LC/NMR using a flow rate of 0.2 mL/min.[6] Problems associated with the implementation of on-line HPLC/NMR have included the need for deuterated solvents, inadequate solvent suppression techniques and low sensitivity.

However, recent rapid advances in HPLC/NMR provide evidence that many of the major technical obstacles have been overcome.[7, 8] With the development of more powerful NMR spectrometers combined with new NMR techniques for solvent suppression it became much easier to obtain well resolved spectra in the on-flow mode. In particular, the solvent suppression technique developed by Smallcombe et al.[9] significantly improves the spectra during the HPLC/NMR run. This experiment which is based ont the WET solvent suppression technique of Ogg et al.[10] combines shaped rf pulses, pulsed-field gradients (PFG), and selective ^{13}C decoupling, and allows to aquire high-quality spectra at on-flow conditions as has been

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shown for the analysis of the chemical composition of polyethylene oxides[8] and the tacticity of oligostyrenes.[11]

Most of the applications of LC/NMR so far are based on isocratic elution techniques and/or the use of partially deuterated solvents. For the separation of random copolymers, however, gradient elution techniques must be applied. In addition, only a very limited number of mobile phases are appropriate for the separation which are not necessarily suitable for on-line NMR detection. In the present study, the analysis of high-conversion random copolymers of styrene and ethyl acrylate will be described. The CCD of these copolymers is very broad and, accordingly, the microstructure is rather complex. The copolymers will be separated by gradient elution on a reversed-phase system. As the mobile phase fully protonated solvents will be used.

Experimental part

Synthesis of poly[(styrene)-co-(ethyl acrylate)]s

Styrene (S, Fluka) and ethyl acrylate (EA, BASF) were destabilized, vacuum-distilled and stored under nitrogen below 0 °C. The copolymer samples were prepared by bulk polymerization at 70 °C under nitrogen using dibenzoyl peroxide (0.025 mol L^{-1}) as the initiator. The reaction mixture was dissolved in ethylacetate and poured into pentane after different reaction times. A series of copolymers having different comonomer concentrations and conversions were prepared similarly. To avoid cross-linking, the samples with the monomer composition of S/EA = 10/90 mol-% was prepared by solution polymerization in toluene. The copolymer samples are listed in Tab. 1, summarizing, the conversions the weight-average molar masses ($\bar{M}_w$) and the average chemical compositions of the copolymers. The molar masses were determined by SEC and the average chemical composition by ^{1}H NMR spectroscopy and elemental analysis.

HPLC

The chromatographic separations were carried out on a Waters LCM 1 module equipped with an evaporative light-scattering detector (ELSD, model 500, Alltech). The column was a Macherey & Nagel Nucleosil RP-18, 5 µm average particle size, 100 Å average pore size, 250 × 4.6 mm I.D. The mobile phase comprised HPLC grade tetrahydrofuran (THF) and acetonitrile (ACN). The mobile phase composition was changed linearly from THF/ACN 10/90 to 100/0 v/v in 25 min Sample concentration was 100 mg/mL in THF/ACN 50/50 v/v, 10 µL of the sample solution were injected.

Tab. 1. Gross compositional analysis of poly[(styrene)-*co*-(ethyl acrylate)]s.

Sample no.[a]	Conversion in wt.-%	$\bar{M}_w$/(g/mol)	C analysis fraction of S in mol-%	^{1}H NMR fraction of S in mol-%
SEA 10/90	95	70 000	10	11
SEA 40/60	98	215 000	40	41
SEA 50/50	96	159 000	50	50
SEA 60/40	97	143 000	60	61
SEA 90/10	99	128 000	90	90

[a] Sample no.: fraction of S/EA in the monomer feed in mol-%.

On-line HPLC/^{1}H NMR

The NMR measurements were conducted on a Varian 500 MHz spectrometer UNITY INOVA™. The HPLC/NMR probe containing a 60 µL flow cell was an indirect detection probe with PFG. All measurements were carried out at room temperature. The following pulse sequence was used: the WET (water suppression enhanced through T1)[9] sequence consisting of four 20 ms selective SEDUCE pulses (98.2, 80.0, 75.0, and 152.2 degrees for the B_1-insensitive WET), four gradient pulses (duration 1 ms) with the amplitudes of 24, 12, 6, and 3 G/cm, respectively, followed by an additional 3 ms delay and a composit 90 degree read pulse. ^{13}C satellite suppression was applied during the selective pulses using band selective WURST decoupling. SEDUCE is a selective pulse which gives highest selectivity for a given pulse length,[12] while WURST is an adiabatic pulse with certain key properties (Wide band, Uniform Rate, Smooth Truncation).[13] For each full spectrum 8 scans were accumulated, the aquisition time for a full spectrum was 9 s.

Results and discussion

Random styrene-ethyl acrylate copolymers are typically complex mixtures which are distributed in molar mass and chemical composition. Depending on the different reactivity of styrene and ethyl acrylate ($r_S/r_{EA} = 0.80/0.20$) in the copolymerization reaction, the instantaneous composition of the copolymer molecules differs from the composition of the monomer mixture. The depletion of styrene in the monomer mixture with increasing conversion causes subsequent portions of the copolymer to be polymerized from monomer mixtures of different compositions. Using appropriate chromatographic conditions, the resulting chemically heterogeneous copolymers can be separated with respect to chemical composition distribution (CCD) in the order of increasing styrene content. As has been shown previously, suitable chromatographic conditions are a C_{18} reversed stationary phase and a linear gradient of THF/ACN as the mobile phase.[14]

The chromatographic separation of a set of styrene-ethyl acrylate copolymers (SEA) according to chemical composition is shown in Fig. 1. The elution order corresponds to the styrene content of the macromolecules, i. e. ethyl acrylate-rich fractions are eluted earlier than styrene-rich fractions. A recalculation of the retention time axis into a composition axis is possible by calibrating the system with chemically uniform samples of known composition. Since such samples are not commercially avail-

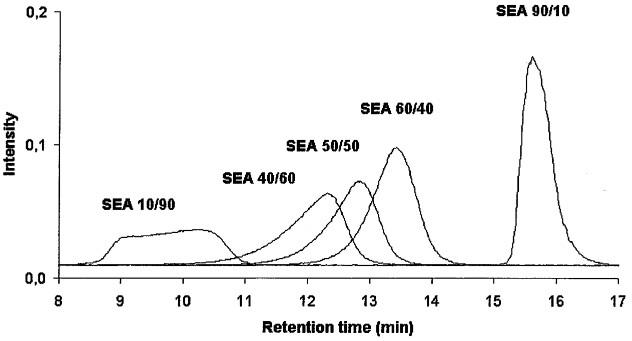

Fig. 1. HPLC-chromatograms of SEA copolymers of different composition obtained with an evaporative light scattering detector; linear gradient ACN/THF 10/90 to 100/0 v/v in 25 min, Nucleosil RP-18 column, flow rate 0.7 mL/min.

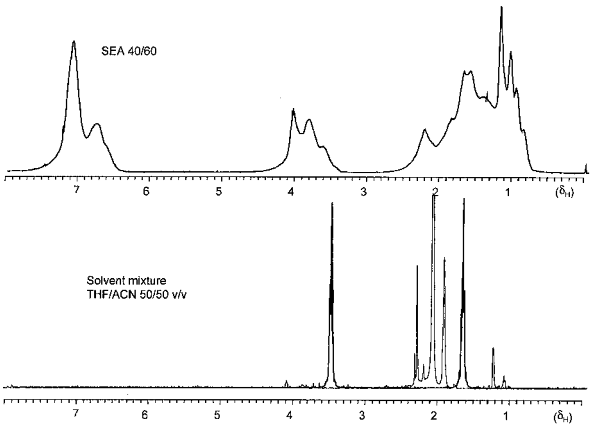

Fig. 2. ^{1}H NMR spectra of a SEA-copolymer (monomer composition S/EA 40/60) and a representative solvent mixture of THF/ACN 50/50 v/v.

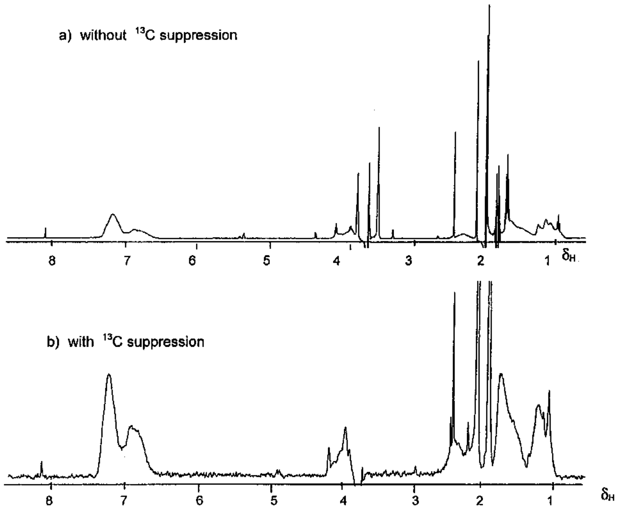

Fig. 3. ^{1}H solvent suppression of SEA 40/60 without (a) and with (b) ^{13}C suppression.

able, they have to be prepared in a rather time-consuming procedure.

In order to avoid unnecessary synthetic work, the chromatographic system shall be coupled to a ^{1}H NMR spectrometer. The chemical composition shall be determined at each point of the copolymer elution curves from characteristic resonance signals of the styrene and the ethyl acrylate moities. The major problem of running an on-line coupled HPLC/NMR experiment is that a very low polymer concentration has to be detected in a solvent mixture which itself yields high intensity resonance signals. Due to the high price of deuterated solvents, in on-line HPLC/NMR protonated solvents are preferably used. Fig. 2 shows the off-line ^{1}H NMR spectra of a SEA copolymer and a 50/50 v/v mixture of THF and ACN which is representative for the mobile phase. As can be seen, the proton signals of ACN at δ_H 2.1 and of THF at δ_H 1.7 and 3.5 overlap with specific resonance regions of the copolymer. In addition, impurities give rise to further signals in the solvent spectrum. For the determination of the chemical composition of the copolymer the aromatic protons of the styrene units at 6.5–7.3 and the methyl protons of the ethyl acrylate units at 0.7–1.3 can be used. These appear to be rather unaffected by the solvent signals.

Several pulse techniques have been introduced to reduce the intensity of the solvent signals. The effective solvent suppression of the present pulse technique is shown in Fig. 3. Due to the gradient elution the composition and the physical behaviour of the solvent is changing, thus solvent suppression must adjust itself to fit to the drift in chemical shift. The LC/NMR microflow probe is configured as a variable temperature, pulsed field gradient ^{1}H-indirect detection probe, and multiple solvent suppression on-the-fly is possible. A one-pulse experiment is used to find the solvent peaks. The transmitter then keeps the biggest solvent peak (here ACN) at a constant frequency and adjusted solvent suppression is applied on the analyte-containing solvent stream. The process is repeated automatically throughout the entire gradient run. In combination with selective excitation, selective ^{13}C decoupling and real-time hardware adjustment allows multiple frequency solvent suppression (referred as WET-NMR) without using presaturation.A series of free induction decays (FID) was collected for each data point, a Fourier transformation via the acquisition times and a combination of the spectra was carried out using the two-dimensional NMR software.

As a result of the on-line HPLC/NMR experiment a contour plot of ^{1}H chemical shift vs. acquisition time is generated, see Fig. 4. The aquisition time is equivalent to the retention time of the HPLC separation. Despite of the significant number of unwanted solvent signals, resulting from impurities of the mobile phase, all important resonance signals of the polymer fraction can be recognized. The projection of all signal intensities towards the aquisition time axis yields an intensity profile which is very similar to the HPLC elution curve detected with a concentration detector.

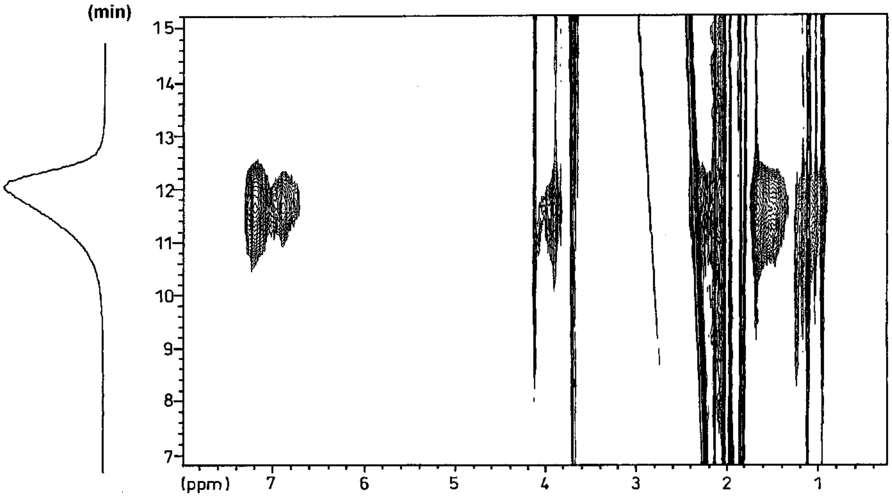

Fig. 4. Contour plot of chemical shift vs. acquisition time of the on-line HPLC/^{1}H NMR analysis of SEA 40/60.

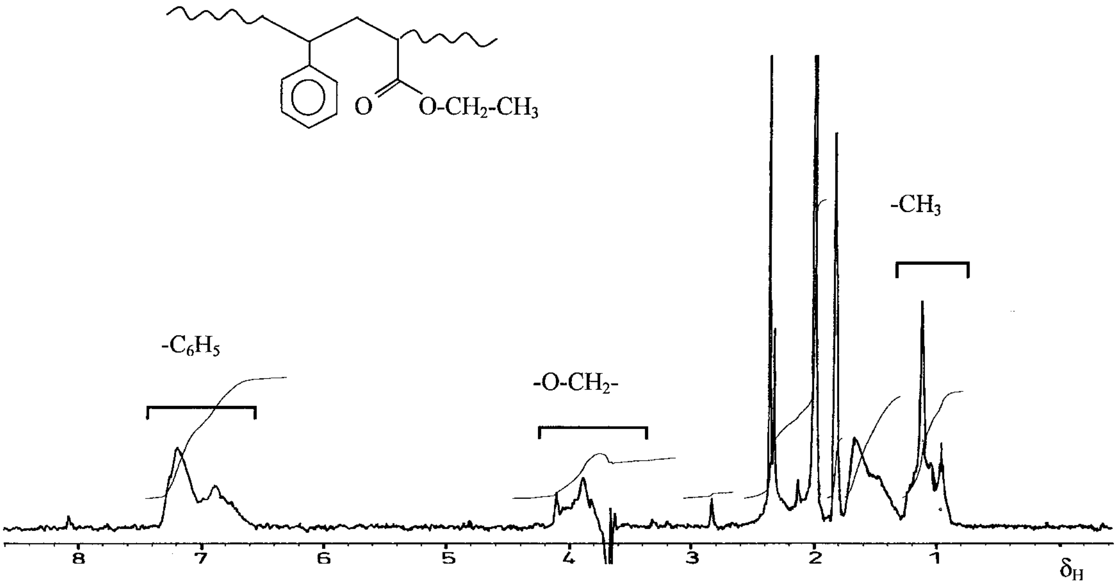

Fig. 5. ^{1}H NMR spectrum of the peak maximum of the HPLC/^{1}H NMR experiment of SEA 40/60.

Fig. 5 shows the NMR spectrum of sample SEA 40/60 extracted at the peak maximum. The peak areas that are used to determine the chemical composition are marked. However, residual signals of the eluent are obtained at 1.8–2.4 and 1.2 due to ACN and impurities. The concentration of the impurity affecting the methyl protons of the ethyl acrylate units at 1.2 is constant during analyte elution, therefore the ethyl acrylate content at each HPLC-fraction could be determined.

Selected spectra of the copolymer SEA 10/90 at different retention times are summarized in Fig. 6. The styrene content is calculated from the intensity of the aromatic protons of the styrene units (δ_H 6.5–7.4) and the methyl protons of the ethyl acrylate units (δ_H 0.9–1.3). In agreement with the properties of the chromatographic system, the styrene content of the fractions increases with increasing retention time. Thus, for a retention time of 558 s a styrene content of 9.6% is calculated, while a retention time of 603 s corresponds to a styrene content of 23.0%.

Using the on-line coupled HPLC/^{1}H NMR procedure, copolymers of different gross composition can be analysed. For different positions at the elution curves the actual styrene content can be determined. As is shown in Fig. 7, the copolymers are separated with respect to chemical composition in the order of increasing styrene content. Each point (–o–) across the elution curves indicates the result of one NMR experiment. For comparison, the chemical composition calibration curve obtained from narrow-disperse copolymer standards is given. As can be seen, a perfect agreement of the two methods is obtained.

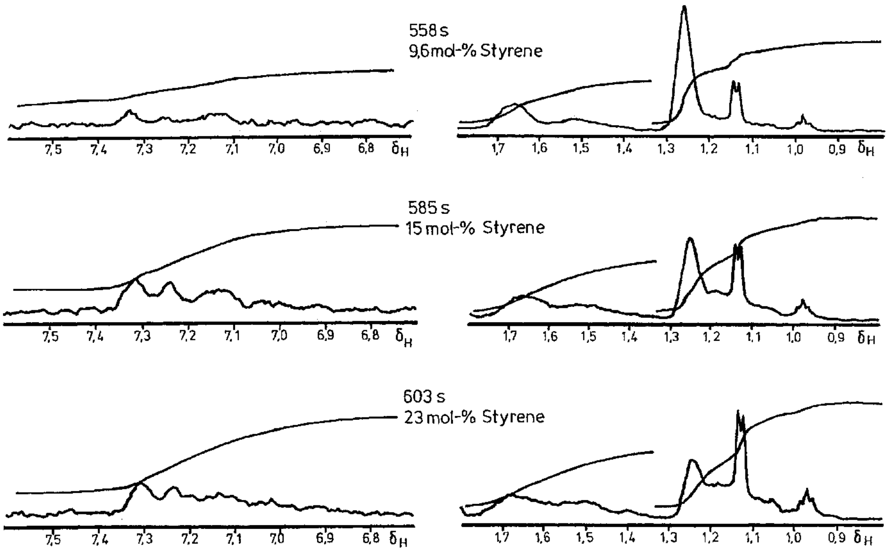

Fig. 6. On-line ^{1}H NMR spectra collection of SEA 10/90 at different retention times and calculated styrene contents.

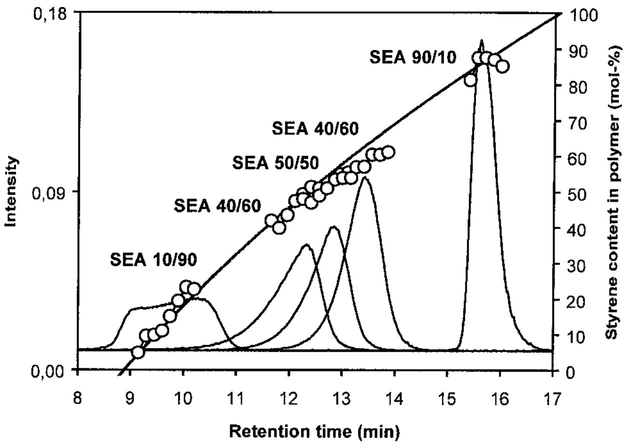

Fig. 7. HPLC-chromatograms of SEA-copolymers of different composition with high conversion. Styrene content obtained by (——) HPLC-calibration; (–o–) on-line HPLC/^{1}H NMR experiments.

To summarize, on-line coupled HPLC/NMR is a unique method for the investigation of the CCD of random copolymers. It is obvious that the gradient HPLC/NMR method is much less time consuming than the conventional off-line calibration. In addition, the NMR detection yields very specific information on the copolymer microstructure at each point of the chromatographic curve.

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