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Investigation of the tacticity of oligostyrenes by on-line h.p.l.c./^{1}H n.m.r.

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Oligostyrenes can effectively be analysed with respect to chemical structure and tacticity by on-line h.p.l.c./^{1}H n.m.r.. The separation of the oligostyrenes into oligomers is conducted on a reversed phase column using acetonitrile as the eluent. Via on-line coupling the chromatographic peaks are directly transferred into the n.m.r. spectrometer and analysed on-flow. Information on the chemical structure of the endgroups, the degree of polymerization and the tacticity of the polymer chain is obtained. The experiments have been conducted under conditions which are common for h.p.l.c. separations, i.e. sufficiently high flow rate, moderate sample concentration and on-flow detection. For the first time, the experiments have been carried out using conventional h.p.l.c.-grade acetonitrile and no deuterium lock.

(Keywords: Coupled H.p.l.c.-n.m.r.; polystyrene; hyphenated techniques)

INTRODUCTION

Nuclear magnetic resonance spectroscopy has proved of exceptional value in the study of the stereochemistry of polymers. With the aid of spectrum-simplifying techniques stereochemically different sequences of up to five monomer units have been distinguished in favourable instances. However, polystyrene has hitherto resisted most attempts to characterize the exact stereochemical structure of the chain. The n.m.r. chemical shifts of protons depend on their magnetic environments which, in turn, depend on the conformations of the neighbouring bonds and, thus, on the stereochemical constitution of the polymer chains. The probabilities of various conformations of the neighbouring bonds depend not only on the stereochemical character of the diad in which the protons are situated, but also in marked degree on the stereochemical character of the neighbouring diads including second and third neighbours and beyond. This long-range dependence is a consequence of the severe conformational constraints generally prevalent in vinyl chains.

As a result, very complex proton as well as carbon-13 n.m.r. spectra are obtained for polystyrene, making it very difficult to interpret them completely. The *ortho*-aromatic proton resonances of polystyrene contain stereosequence information but these are not adequately understood at present[1,2]. The methine proton resonances occur in two general areas, and it has been shown in studies on partially epimerized isotactic polystyrene that the lower field methine proton resonance area is due to mm stereosequences[3]. Fine structure due to pentad or higher stereosequences has been observed in the methine proton resonances of partially deuterated polystyrene[4,5]. The methylene and quaternary aromatic carbon resonances are sensitive to hexad and heptad stereosequence effects[6,7]. The abundant information available in the ^{13}C n.m.r. spectra of polystyrene cannot be determined precisely, because the individual resonances cannot be resolved and because of the difficulty in making unequivocal assignments for the various resonances. The detailed analysis of the methylene and phenyl C-1 carbons of polystyrene was reported by Sato and Tanaka[8]. According to them, the most important signal for determining stereoregularity is the phenyl C-1 carbon signal[8]. Comparing atactic, isotactic and syndiotactic polystyrene, Ishihara *et al.*[9] reported that the spectrum of isotactic polystyrene shows a single sharp peak at about 146.24 ppm corresponding to the mmmm pentad configuration, whereas in syndiotactic polystyrene a single sharp peak at 145.13 ppm is due to an rr triad or rrrr pentad configuration. Atactic polystyrene shows five main peaks in the range 145.12–146.7 ppm corresponding to its various configurational sequences[9].

A different approach was reported by Sato and co-workers in a number of papers[10–12]. Using g.p.c. they separated anionically polymerized polystyrene into single oligomers and then subjected the oligomers to multiple h.p.l.c. separations to obtain individual steric isomers. In some cases more than 50 cycles of separation were carried out, making the procedure an extensively time-and labour-consuming technique. The isolated isomers were then subjected to proton and ^{13}C n.m.r. analysis.

The combination of h.p.l.c. with nuclear magnetic resonance has been attempted numerous times. Early experiments of coupled h.p.l.c./^{1}H n.m.r. were conducted in a stop–flow mode or with very low flow rates[13–15]. 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.*[16] described the analysis of isotactic poly(methyl methacrylate) by on-line l.c./n.m.r. using a flow rate of 0.2 ml/min. Problems associated with the implementation of on-line h.p.l.c./n.m.r. have included the need for deuterated solvents, inadequate solvent

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suppression techniques and low sensitivity. However, recent rapid advances in h.p.l.c./n.m.r. provide evidence that many of the major technical obstacles have been overcome[17,18].

With the development of more powerful n.m.r. spectrometers combined with new n.m.r. techniques for solvent suppression it became much easier to obtain well-resolved spectra in the on-flow mode. In particular, the solvent suppression technique recently developed by Smallcombe *et al.*[19] significantly improves the spectra during the h.p.l.c./n.m.r. run. This experiment which is based on the WET solvent suppression technique of Ogg *et al.*[20] combines shaped RF pulses, pulsed-field gradients (PFGs), and selective ^{13}C decoupling, and allows one to acquire high-quality spectra at on-flow conditions even with high h.p.l.c. gradients. Based on this new technique the present study describes the analysis of oligostyrenes by

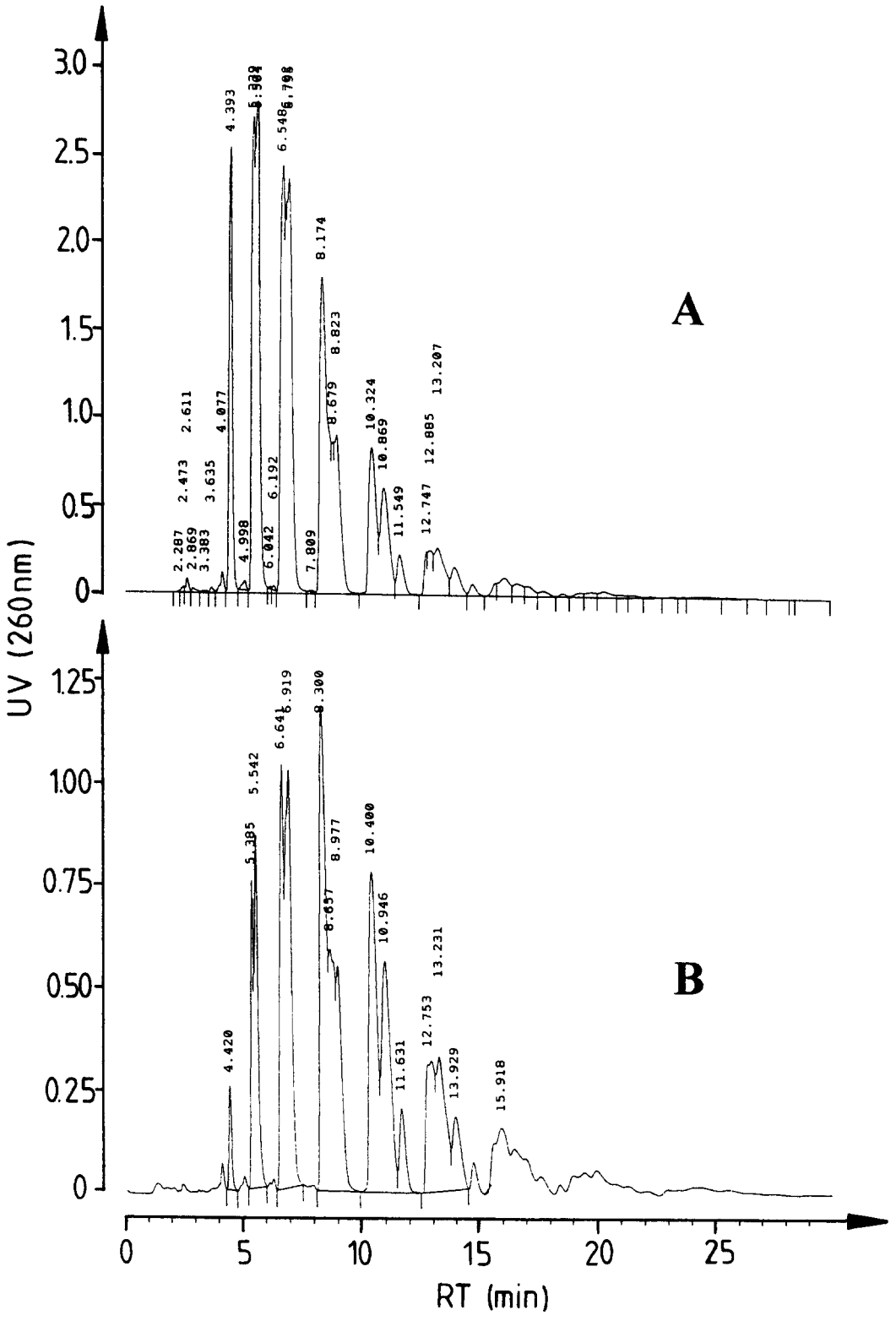


Figure 1 Chromatographic separation of two oligostyrenes with average molar masses of 530 (A) and 690 g/mol (B). Chromatographic conditions: stationary phase, Nucleosil 100-5 C_{18}; eluent, acetonitrile

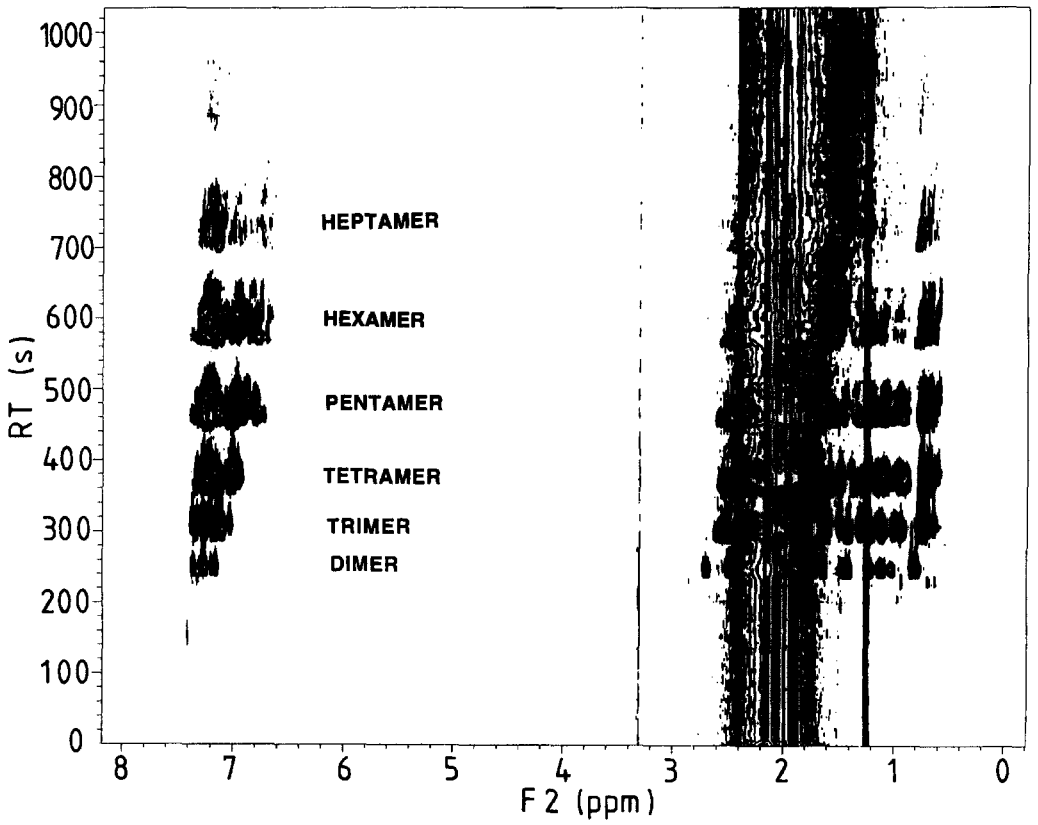


Figure 2 Contour plot of chemical shift *versus* retention time of the on-line h.p.l.c./n.m.r. analysis of PS 530

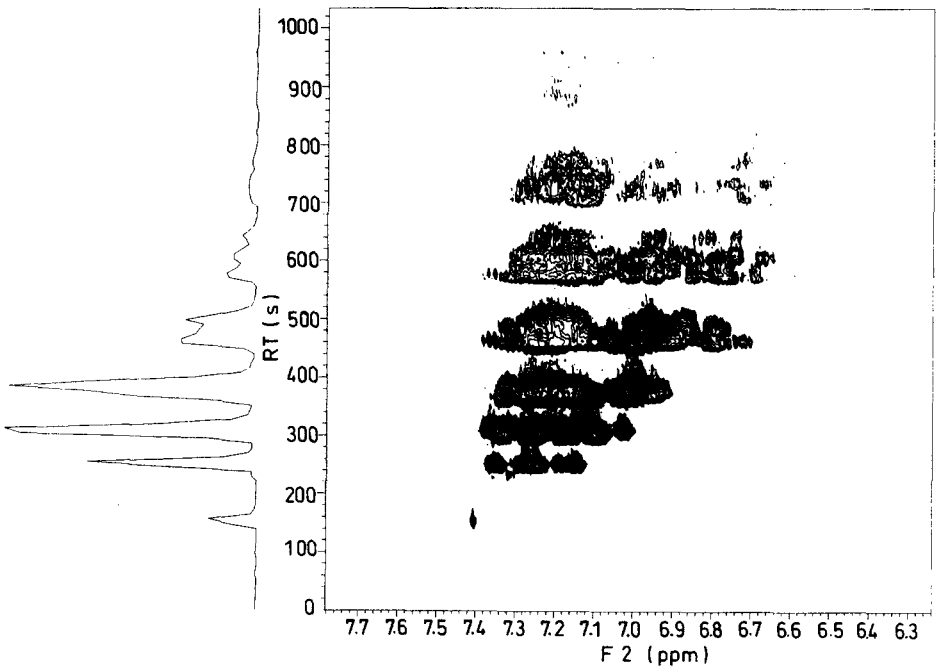


Figure 3 Contour plot of chemical shift *versus* retention time of the aromatic region of the on-line h.p.l.c./n.m.r. analysis of PS 530

h.p.l.c./n.m.r. with respect to chain length and tacticity. Emphasis has been put on the use of experimental conditions which are common in h.p.l.c. regarding flow rate and sample concentration.

EXPERIMENTAL

Sample

The oligostyrenes were g.p.c. calibration standards, prepared by anionic polymerization using *sec*-butyllithium as the initiator. They were provided by Polymer Standards Service Mainz, Germany.

H.p.l.c.

The chromatographic separations were carried out on a Varian modular h.p.l.c. system, comprising a Varian 9012 pump, a Valco injection valve and a Varian 9050 u.v. detector. The u.v. detector was operating at a wavelength of 260 nm. The column was a Macherey and Nagel Nucleosil RP-18, 5 μm average particle size, 100 Å average pore size, 250 × 4 mm i.d. The mobile phase was h.p.l.c.-grade

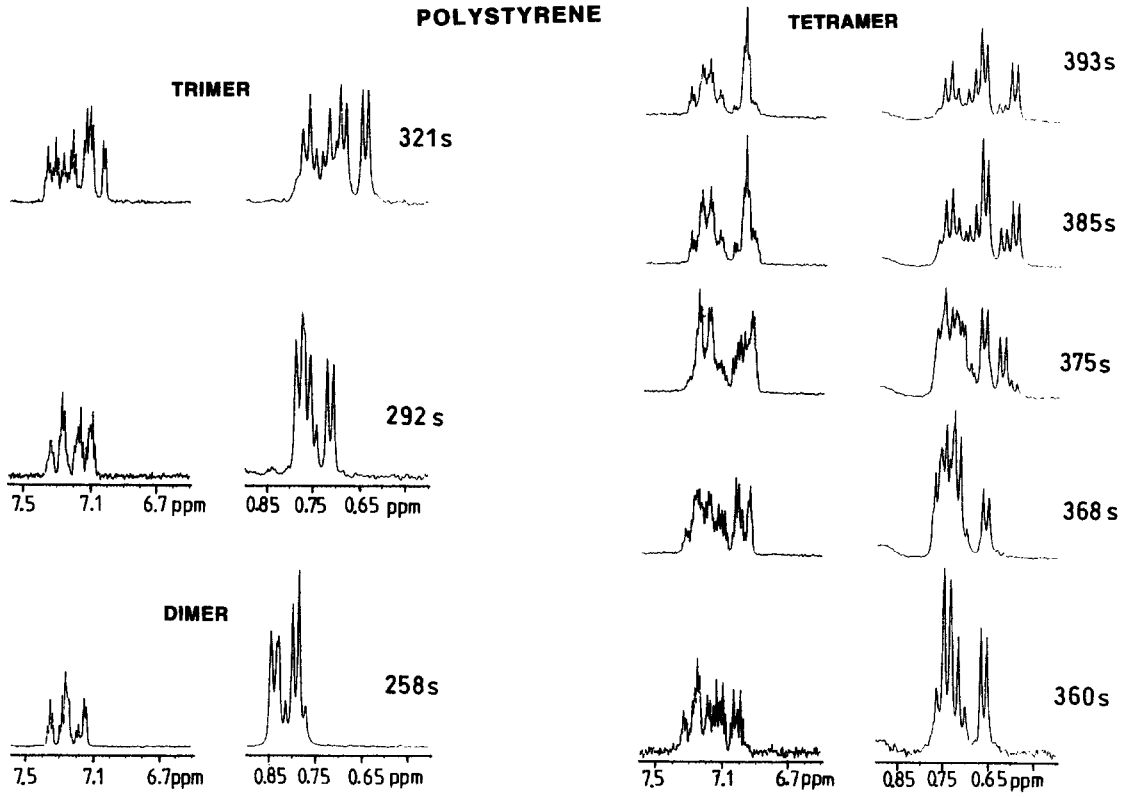


Figure 4 Aromatic and methyl proton regions of different oligomer peaks of PS 530, obtained from the coupled h.p.l.c./n.m.r. experiment

acetonitrile. The sample concentration was 15 mg/ml in acetonitrile, 100 μl of the sample solution were injected. The flow rate was changed according to the following programme (time/flow rate): 0/1.00, 8/1.00 linear to 20/2.00, 25/2.00 linear to 30/1.00 min/ml/min.

N.m.r.

The n.m.r. measurements were conducted on a Varian 500 MHz n.m.r. spectrometer UNITY*plus*. The h.p.l.c./n.m.r. probe containing a 60 μl flow cell was an indirect detection probe with PFG. All measurements were carried out at room temperature.

RESULTS AND DISCUSSION

The different techniques of liquid chromatography offer a wealth of procedures which can be used to separate oligomers and polymers with respect to molar mass, chemical composition or functionality. In particular, polymers with moderate and low molar masses can be separated into single oligomers by gel permeation chromatography (g.p.c.), as has been shown for poly(methyl methacrylate) by Andrews and Vatvars[21]. About 40 individual oligomers could be isolated from a polystyrene calibration standard by isocratic elution from a silica gel column using *n*-pentane/tetrahydrofuran as the eluent[22]. With two silica gel columns and a gradient of *n*-hexane/dichloromethane the fine structure of the first peaks could be revealed and attributed to stereoisomers[23].

A rather simple separation scheme for oligostyrenes was used in the present investigation. Using isocratic elution with acetonitrile on a reversed phase column RP-18, similar to the procedure of Eisenbeiss *et al.*[24], separation into tactic isomers was also obtained. In order to reduce the elution time, a flow rate gradient was used. Starting with a flow rate of 1 ml/min, after 8 min of isocratic elution the flow rate was increased linearly to a value of 2 ml/min at 20 min. This flow rate was kept for 5 min, and then, at a retention time of 25 min, the flow rate was decreased linearly to 1 ml/min within 5 min to re-establish the initial chromatographic conditions.

The chromatograms of two oligostyrenes are shown in *Figure 1*. The samples were prepared by anionic polymerization using butyllithium as the initiator. For both samples a separation into oligomers of different chain lengths is obtained. The first oligomer peak appears at a retention time of about 4.4 min. By comparison with the individual oligomer, this peak could be identified as being the dimer (degree of polymerization $n = 2$). The next peak at about 5.4 min was identified as the trimer ($n = 3$) and, accordingly, the following peaks could be assigned to the tetramer, pentamer, etc. The dimer peak appears uniform, whereas for the following oligomers a splitting of the peaks is obtained. For $n = 3$ and $n = 4$ a splitting into two peaks is observed. For $n = 5$ and further a splitting into three or more peaks occurs.

In agreement with previous investigations, the resolution of more than one peak for a single oligomer is a result of the formation of different tactic isomers. For structural identification of the isomerism of the oligomers, h.p.l.c. is coupled on-line to the n.m.r. spectrometer. The major problem in studying styrene oligomers by h.p.l.c./n.m.r. is that for the h.p.l.c. separation only organic solvents are used. N.m.r. prefers deuterated solvents which are very expensive and it will be demonstrated that high quality h.p.l.c./n.m.r. experiments can also be performed without adding any deuterated component. In other words, no deuterium lock was used for the on-flow measurements. All measurements were conducted in normal h.p.l.c.-grade acetonitrile. These conditions require high stability of the n.m.r. instrument and

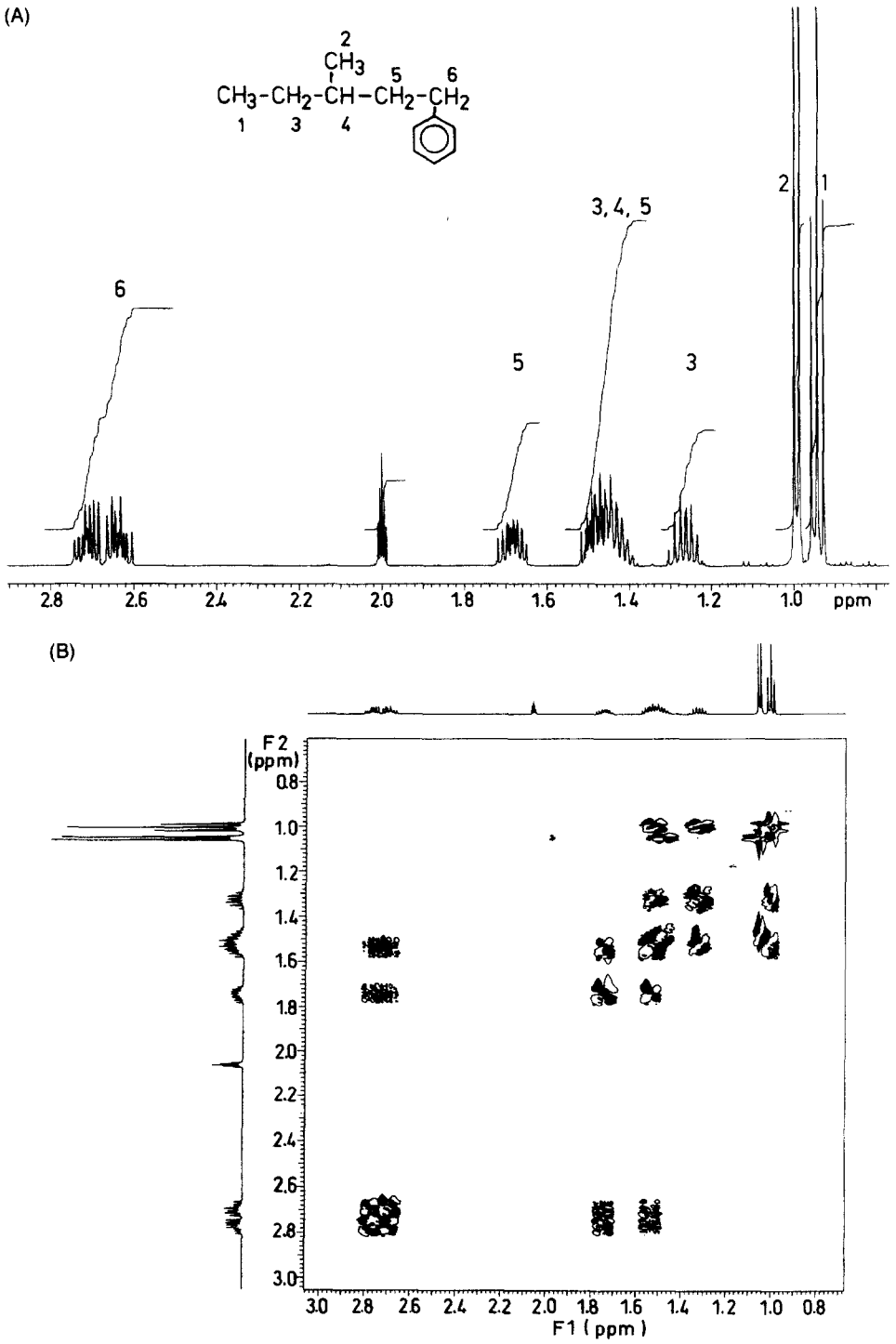
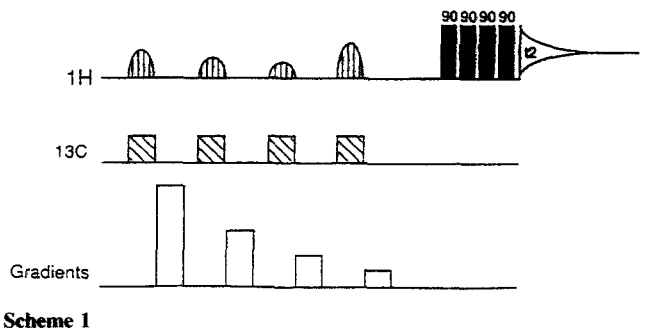


Figure 5 ^{1}H n.m.r. spectrum (A) and gradient DQF COSY spectrum (B) of the styrene monomer



Scheme 1

a very efficient solvent suppression technique since 100% acetonitrile must be suppressed. The following pulse sequence was used, see *Scheme 1*[25]: the WET sequence applied to h.p.l.c./n.m.r. consisting of four 20 ms selective SEDUCE pulses (98.2, 80.0, 75.0 and 152.2° 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 composite 90° read pulse. Carbon decoupling was applied during the selective proton pulses using Waltz-16 decoupling.

After leaving the u.v. detector, the eluate is introduced directly into the n.m.r. cell via capillary tubing. Since a series of free induction decays (FID) was collected, a Fourier transformation via the acquisition times and

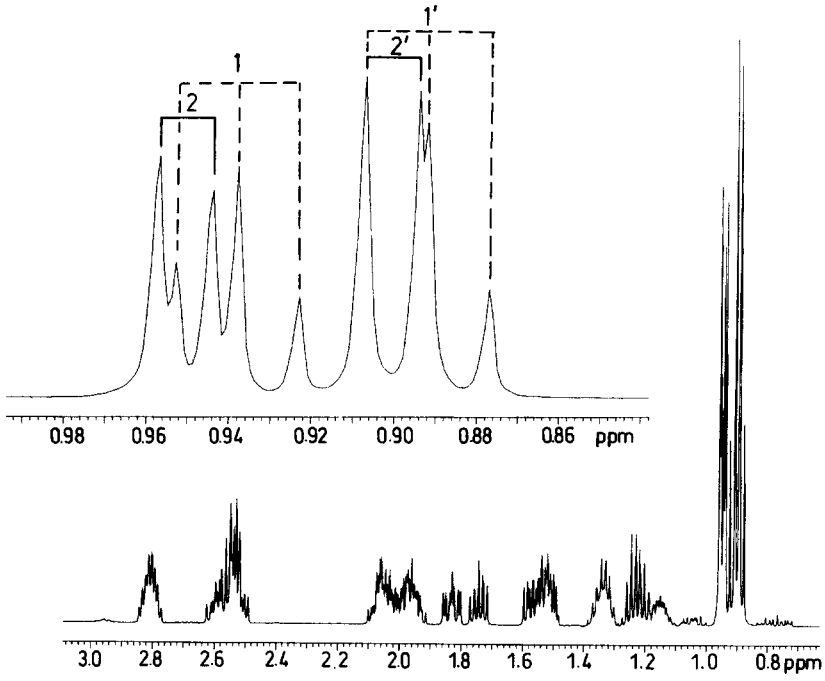


Figure 6 ^{1}H n.m.r. spectrum of the styrene dimer

a combination of the spectra could be carried out regarding the two-dimensional n.m.r. software. As a result of the on-line h.p.l.c./n.m.r. experiment a contour plot of ^{1}H chemical shift *versus* retention time can be generated, see *Figure 2*. The obtainable structural information relates to the entire chemical shift region; however, residual signals of the eluent are obtained at 1.8–2.4 and 1.3 ppm due to acetonitrile and its impurities.

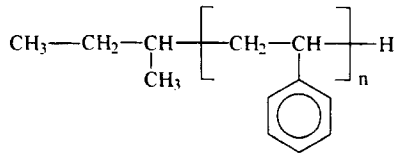
The contour plot clearly reveals two signal regions, which can be used for analysis. These are the region of the methyl protons of the *sec*-butyl endgroup at 0.6–0.8 ppm and the aromatic proton region of the styrene units at 6.5–8.0 ppm. The contour plot for the aromatic region, see *Figure 3*, shows that systematic changes of the signals occur with changes in the degree of polymerization. For the dimer, the aromatic proton signals are located in the narrow range of 7.1–7.4 ppm, whereas for the pentamer the signals are distributed over the range 6.6–7.1 ppm. The projection of the aromatic signals on the retention time axis results in a chromatogram presentation, which is fairly similar to the initial chromatogram, shown in *Figure 1*.

For the generation of the contour plot every 8 s a complete spectrum is produced by coadding eight scans. Accordingly, for the structural analysis 128 spectra are available over the entire retention time range. For the analysis of a separated oligomer, a minimum of four spectra can be used. These spectra bear selective information on the tacticity, even without completely separating the tactic isomers chromatographically.

The spectral characteristics of the aromatic and methyl proton regions of the different oligomers are summarized in *Figure 4*. For the dimer, in total four spectra are obtained which are completely similar, indicating that the dimer is present only in one isomeric form. However, assuming one isomeric form, for the methyl protons the appearance of a doublet and a triplet must be expected, since the chemical structure of the endgroup is CH_3–CH_2–$CH(CH_3)$–. Instead, two doublets and two triplets are obtained, which clearly show that both isomeric forms are present. The resolving power of liquid chromatography is in this case not sufficient for a separation.

The trimer gave four spectra, two of them revealing different isomeric structures. The other two spectra turned out to result from overlapping of the isomer spectra. Again, each of these spectra show two doublets and two triplets, and thus relate to two different isomeric structures. Accordingly, for the trimer the expected four different isomeric forms are present. For the tetramer five different spectra are obtained, and here it is very difficult to judge which of the spectra is an overlap. Nevertheless, the different spectra appear due to the iso-, syndio- and atactic configurations. The pentamer gave 11 different spectra, but because of their complexity an identification of isomers seems to be impossible.

Since the spectral information of the h.p.l.c./^{1}H n.m.r. experiment is very complex and the interpretation is not straightforward, additional off-line experiments were carried out. For the n.m.r. analysis of individual oligomers, an oligostyrene sample was fractionated by distillation, and the monomer, dimer and trimer were isolated. The following structure can be assumed for these species:



n = 1, 2, 3

The aliphatic region of the ^{1}H n.m.r. spectrum of the monomer is shown in *Figure 5A*. The assignment of the signals can be made by gradient double quantum filtered (DQF) COSY and gradient heteronuclear single quantum coherence (HSQC) experiments. As can be clearly seen, for the methyl protons of the *sec*-butyl endgroup a doublet at about 1 ppm and a triplet at 0.6–0.8 ppm are obtained. The doublet couples with the methine proton in position 4 and can be assigned to the methyl group 2. The triplet couples with the methylene protons in position 3 and,

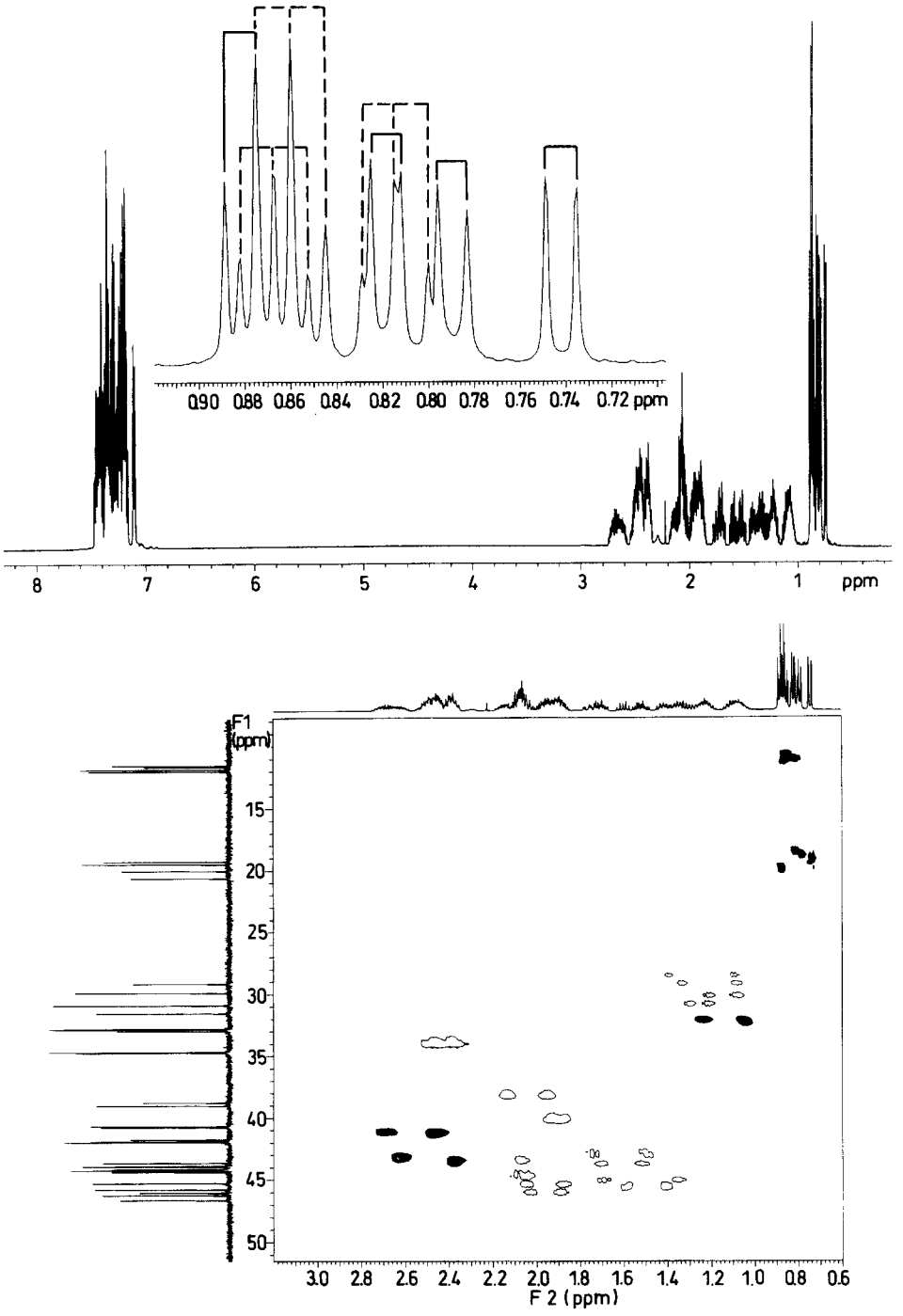


Figure 7 ^{1}H n.m.r. spectrum (A) and $^{1}H-^{13}C$ correlation (gradient HSQC with carbon multiplicity edition) spectrum (B) of the styrene trimer: full crosspeaks, CH_3 or CH; empty crosspeaks, CH_2

accordingly, is assigned to the methyl group 1, see *Figure 5B*.

A much more complicated spectrum results from the measurement of the dimer, being the first oligomer peak in *Figure 1*. For the methylene and methine protons complex multiplets are obtained which shall not be analysed in the present article. An interesting pattern is obtained for the protons of the methyl groups 1 and 2, see *Figure 6*. Nine signals appear in this region which are identical to the signals of the dimer peak in *Figure 4*, obtained by the on-line h.p.l.c./n.m.r. experiment. These signals can be grouped into two doublets and two triplets as is shown in the zoomed part of *Figure 6*. The appearance of two doublets for methyl group 2 and two triplets for methyl group 1 is clear evidence for the presence of two isomeric structures. Additional evidence is provided by the ^{13}C n.m.r. spectrum, where four methyl carbon signals appear instead of two. In agreement with Ishihara *et al.*[9] the lower field doublet and triplet is assigned to the isotactic isomer, whereas the syndiotactic isomer shows signals at higher field.

The interpretation of the spectral features of the trimer is only possible by combination of different measurements. The proton n.m.r. spectrum is too complex to extract directly any structural information, see *Figure 7A*. However, the methyl group pattern in the range 0.72–0.90 ppm indicates that more than two doublets and triplets are present. This confirms our assumption that the trimer forms more than two isomeric structures. Theoretically, the following four tactic isomers can be encountered.

The $^{1}H-^{13}C$ correlation experiment is in perfect

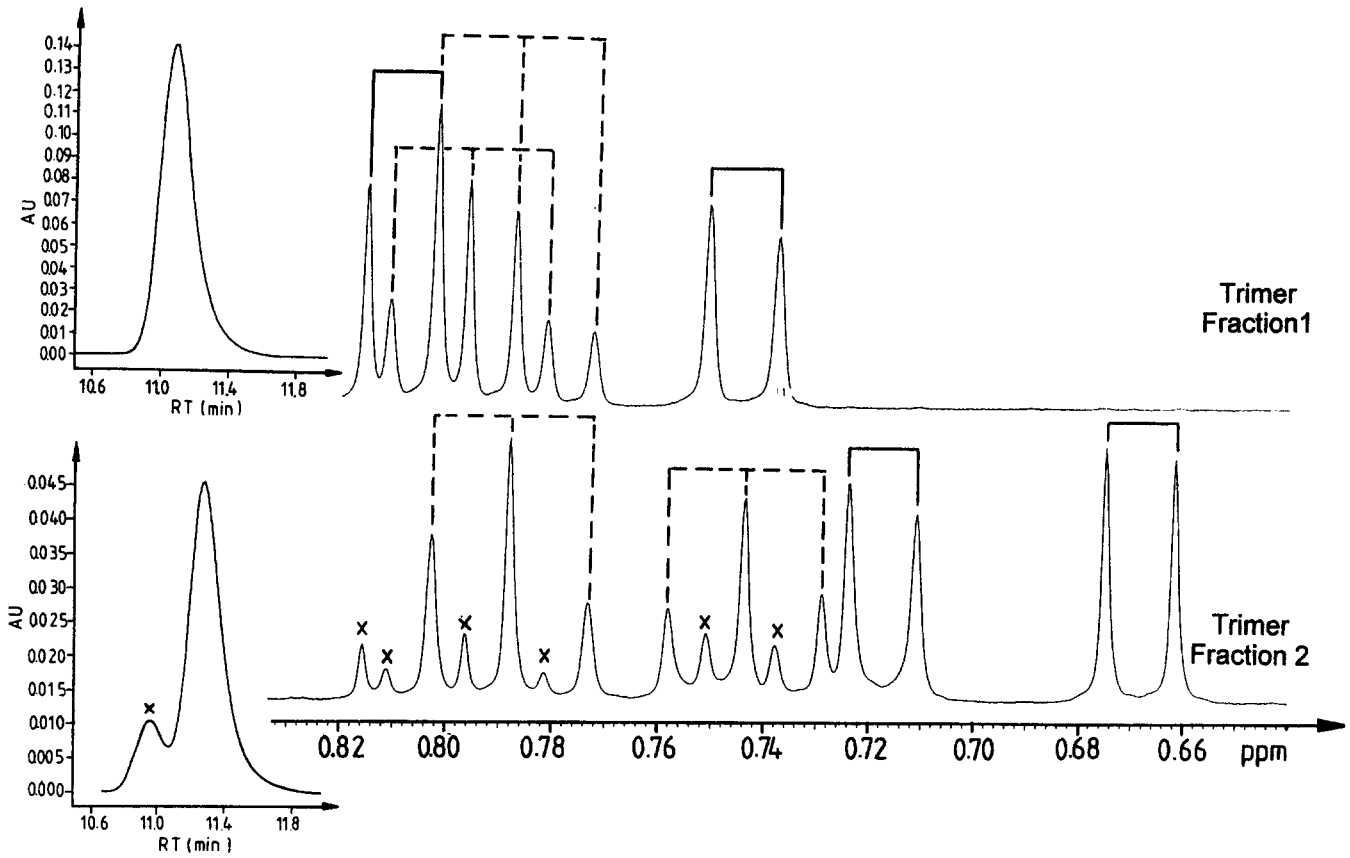
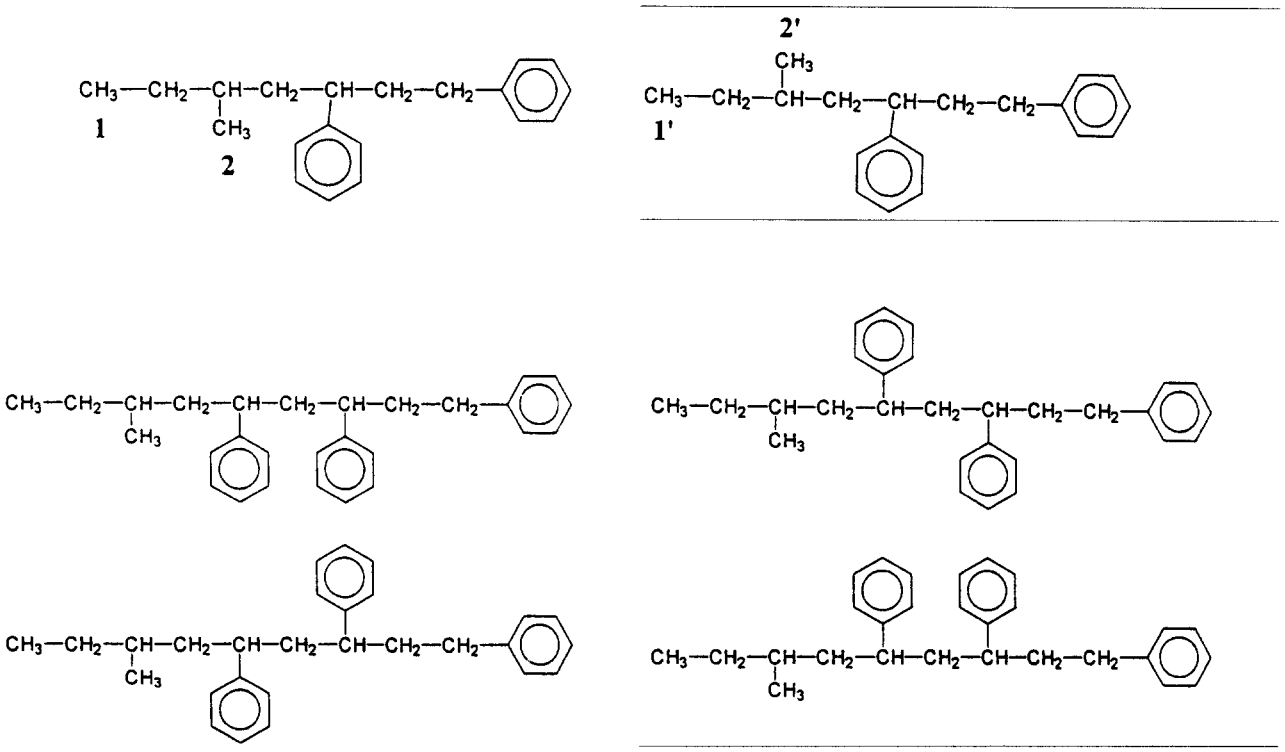


Figure 8 Chromatograms and ^1H methyl group regions of fractions 1 and 2 of the chromatographic separation of the trimer



agreement with this assumption, showing four signals for each methyl group, see *Figure 7B*.

As has been shown in *Figure 1*, h.p.l.c. separates the trimer into two peaks instead of the theoretically expected four isomers. It is, therefore, possible that each peak contains two isomers. Since it can be assumed that the n.m.r. analysis of mixtures of two isomers is easier compared to a complex four-isomer mixture, the trimer was separated into two fractions by preparative h.p.l.c. The fractions were then subjected to a proton n.m.r. analysis. *Figure 8* shows

that fraction 1 is rather pure whereas fraction 2 contains a small impurity of fraction 1. As was expected, the ^{13}C spectra of the fractions show a splitting into two peaks for each methyl group, indicating that each peak contains two isomers. The methyl group region of the proton n.m.r. spectra of the fractions, summarized in *Figure 8*, exhibits a much lower complexity compared to the corresponding spectrum for the total trimer given in *Figure 7A*. Here it is rather straightforward to identify two doublets and two triplets in each spectrum, corresponding to the respective isomeric structures. The signals in fraction 2 indicated by (x) are due to impurities from fraction 1. The assignment to doublets and triplets in fractions 1 and 2 can then be used to assign the signals in *Figure 7A* to the corresponding doublets and triplets. One triplet appears at similar chemical shifts in fractions 1 and 2 and, accordingly, in the trimer spectrum four doublets and only three triplets are found. The assignment of the spectral features to the corresponding tactic isomers cannot be obtained directly from the analysis of the dimer. This is possible only by direct comparison with a pure isotactic trimer.

Comparing *Figures 4, and 8* it is obvious that the on-line experiment already revealed the best obtainable information. The peak patterns from the off-line measurements of fractions 1 and 2 (*Figure 8*) are in perfect agreement with the n.m.r. spectra for the trimer at 292 and 321 s (*Figure 4*), respectively. As has been shown for the current chromatographic conditions, the on-line experiment always gives one well-resolved n.m.r. spectrum as an overlap of two isomers. Accordingly, the five different spectra obtained for the tetramer (*Figure 4*) relate to four well-resolved isomer spectra, each of them being the result of the overlap of two isomers. This results in a total number of eight isomeric structures for the tetramer, as has to be expected.

To summarize, on-line h.p.l.c./^{1}H n.m.r. is a unique method for the investigation of the tacticity of oligostyrenes. By h.p.l.c. a separation into single oligomers can be carried out; in addition, h.p.l.c. provides for the separation into groups of isomers. By n.m.r. the chromatographic peaks can be analysed with respect to the chemical structure (end-groups and chain length) and tacticity. In the case where tactic isomers are not separated by h.p.l.c., the overlapping signal patterns can be used for identification. The assignment of a certain signal pattern to a specific tactic isomer, however, is complicated and will be dealt with in a forthcoming publication.

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