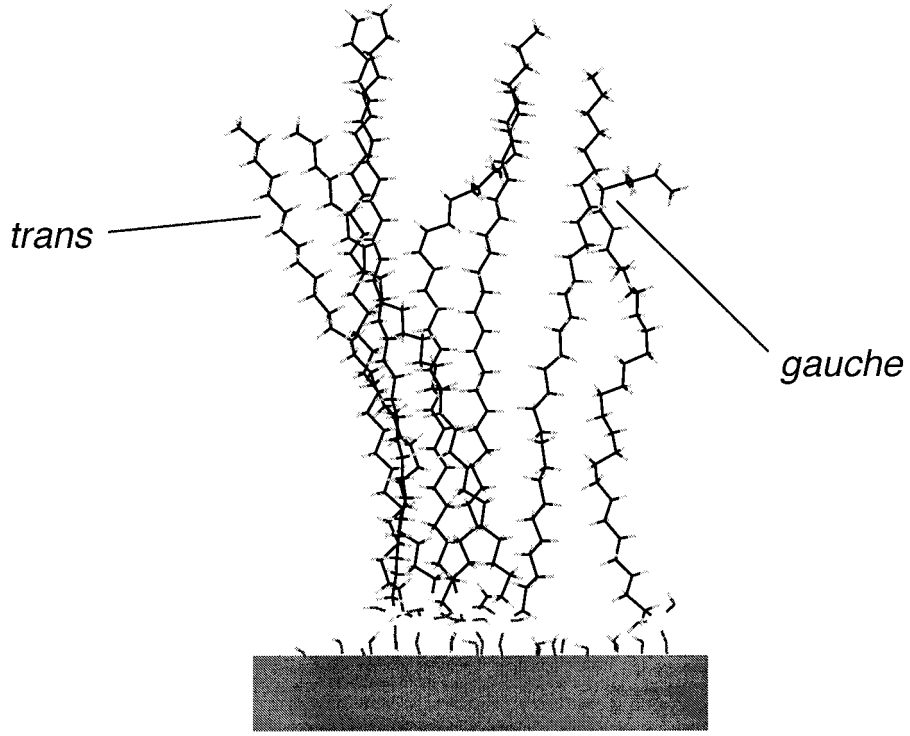

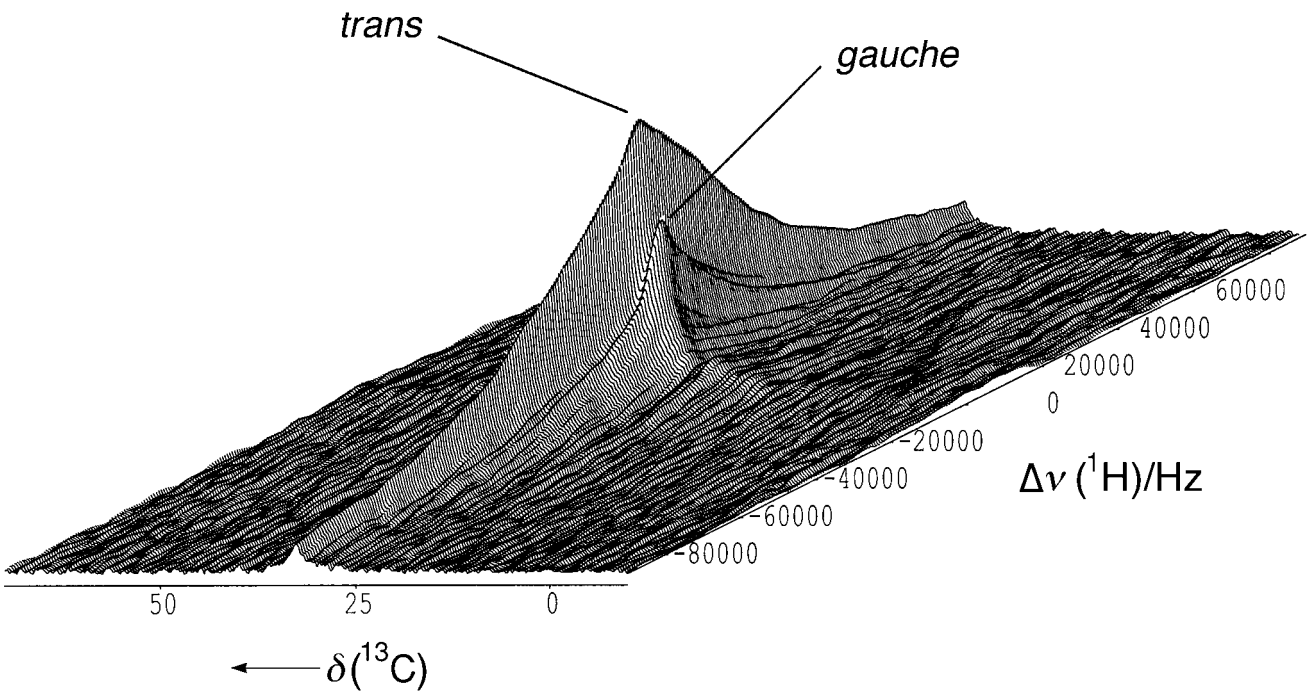

The dynamic behavior of the alkyl chains of a stationary C_{30} interphase for HPLC can be characterized with a 2D solid-state NMR spectrum. Find out more about the application of this method on the following pages.

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Investigating the Selectivity of Triacontyl Interphases**

Klaus Albert,* Tanja Lacker, Martin Raitza, Matthias Pursch, Hans-Joachim Egelhaaf, and Dieter Oelkrug

In addition to the use of octyl- and octadecyl-modified silica gel (C_8 and C_{18} bonded phases) for the separation of isomeric mixtures by reverse-phase high-performance liquid chromatography (RP-HPLC), there is a progressive application of separation phases with triacontyl groups (C_{30} bonded phases). Owing to their specific selectivity, these materials are now widely employed especially for the analysis of carotenoids and tocopherol derivatives.[1–7] We present here an investigation of the structural parameters and interactions responsible for the specific separation behavior. The selectivity (selectivity factor α) of a chromatographic separation is dependent upon the interactive game of the stationary and mobile phases with the different analytes. Within this complicated interphase system, the mobile phase penetrates into the stationary component of the alkyl-modified silica gel in molecular dimension without forming a homogeneous solution. The different partition coefficients between mobile and stationary phases are responsible for the chromatographic separation within different components of a mixture.

Important parameters of the chromatographic interphase system are the structure and dynamics of the stationary phase, the composition of the mobile phase, and the steric and electronic structure of the analyte. To investigate the dynamic behavior of these complex systems, solid-state NMR, suspended-state MAS NMR (MAS = magic angle spinning), and fluorescence spectroscopies were combined with HPLC separations at various temperatures.

The investigated C_{30} interphase was prepared by the solution polymerization procedure, in which the partially hydrolyzed silane is tethered to the silica surface after partial polycondensation.[1, 4] The specific arrangement of the *n*-alkyl groups provided is reflected by the behavior of solid-state ^{13}C NMR spectra measured at various temperatures (Figure 1). Two clearly separated signals at $\delta = 30.0$ and 32.8 are observed for the carbon atoms of the methylene main chain at 305 and 315 K. At lower temperatures the low-field resonance is more intense, whereas the intensity of the high-field resonance increases with higher temperatures. According to solid-state ^{13}C NMR investigations of polyethylene, the signal at $\delta = 32.8$ is assigned to ordered alkyl-chain domains with *trans* conformations and that at $\delta = 30.0$ to more disordered domains

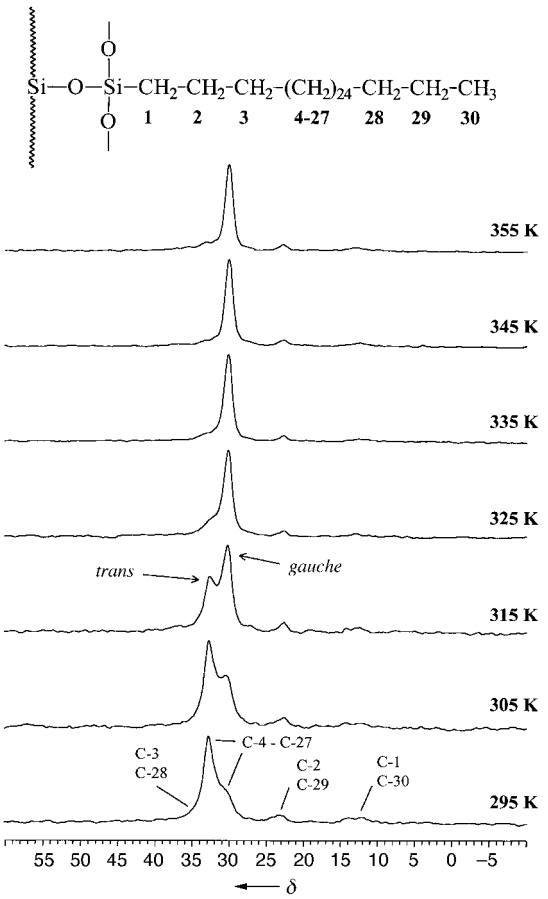

Figure 1. Temperature dependence of the ^{13}C CP/MAS NMR spectra (75.4 MHz) of the C_{30} phase.

with *gauche* conformations.[8] The described signal assignment can be checked with a two-dimensional wideline separation (WISE) experiment.[9] In Figure 2a the ^{13}C chemical shifts are plotted against the half-height widths $\Delta\nu$ of the proton signals. It is clearly evident from Figure 2b that the half-height widths for protons in *trans* conformations ($\delta(^{13}C) = 32.8$) is much larger (48 kHz) than that for protons in *gauche* conformations ($\delta(^{13}C) = 30.0$; 15 kHz). The half-height width for the signal of a methylene proton ensemble is reciprocal to the spin–spin relaxation time T_2 of the protons involved and thus to the averaged overall mobility. Therefore, more immobile *trans* conformations can unambigously be distinguished from flexible *gauche* conformations.

Both conformations of the *n*-alkyl chains can be interconverted by changing the temperature, as indicated in Figure 1. At 295 K mainly *trans* conformations exist beside minor amounts of *gauche* conformations, whereas above 335 K *gauche* conformations appear exclusively. Moreover, in the presence of a mobile phase—the medium in which the chromatographic separation takes place—*gauche* and *trans* conformations of the C_{30} alkyl chains can be detected by suspended-state MAS NMR spectroscopy.[7] The relative amounts of each conformation vary according to the composition of the mobile phase. *tert*-Butyl methyl ether (TBME), which is often used as a modifier in HPLC separations, leads to a preference for *gauche* conformations and thus increases the overall mobility of the alkyl chain.[7]

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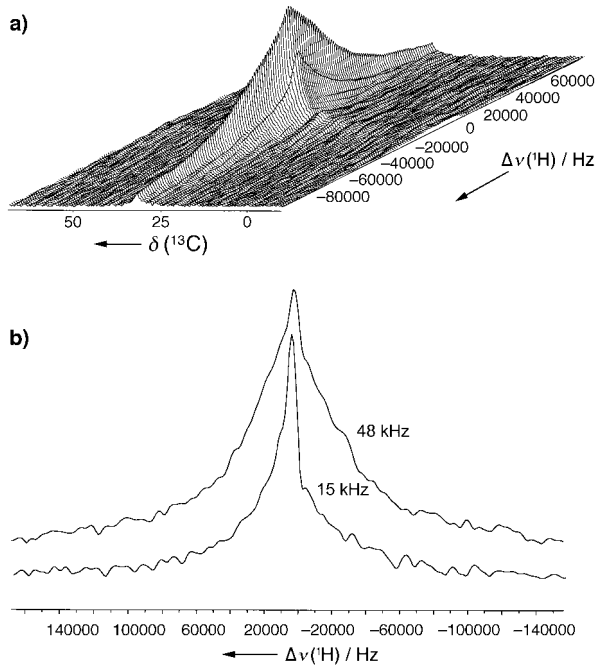

Figure 2. a) 2D WISE NMR spectrum of the C_{30} phase. b) ^{1}H wide-line spectra ($\Delta\nu$ in Hz) derived from the 2D WISE NMR spectrum of resonances for the *trans* and *gauche* conformations.

Suspended-state ^{13}C NMR spectra of a C_{30} phase were recorded at various temperatures (Figure 3) in methanol/TBME (75/25, v/v); this solvent mixture is preferably used for separating β-carotene isomers. At 295 K, the relative amounts of *trans* and *gauche* alkyl-chain conformations are basically the same as indicated by the solid-state NMR spectra. In analogy to the behavior observed in the solid-state NMR spectra, the population of *trans* and *gauche* conformations interchanges with increasing temperature (Figure 3).

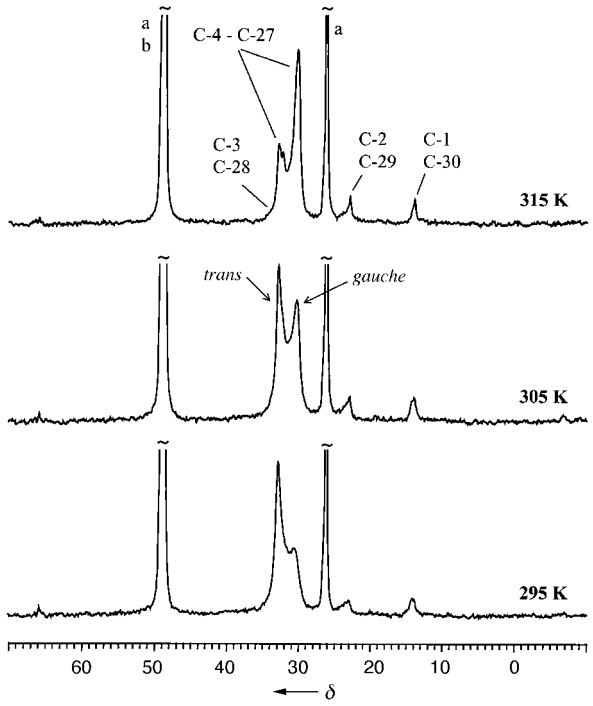

Figure 3. Temperature dependence of the suspended-state ^{13}C MAS NMR spectra (75.4 MHz) of the C_{30} phase in methanol/TBME (75/25, v/v). Solvent signals are indicated with a (TBME) and b (methanol).

A change in the arrangement of the alkyl chain, caused by an alteration in the composition of the mobile phase or temperature, should result in a different dynamic and chromatographic retention behavior of the analyte. In the case of analytes containing a fluorophoric group, the dynamic behavior in the interphase system can be determined from the rotation correlation time τ_R. This parameter is obtained from fluorescence anisotropy measurements.[10] The reciprocal value of the rotation correlation time τ_R is proportional to the rotation diffusion coefficient of the employed fluorescence probe and directly monitors the mobility in the interphase system.[10] Figure 4 clearly shows that the analyte employed, 1,6-diphenylhexatriene (DPH), increases its mobility with increasing temperature in the interphase system consisting of the C_{30} separation phase and acetonitrile/water as mobile phase. The average rotation correlation time $\bar{\tau}_R$ shown in Figure 4 were calculated from stationary fluorescence anisotropies and the corresponding fluorescence decline curves τ_F.

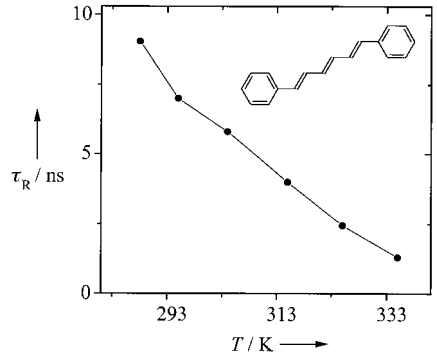

Figure 4. Temperature dependence of the average rotation correlation time $\bar{\tau}_R$ of DPH in the interphase system C_{30} phase – DPH – acetonitrile/water (50/50, v/v).

The overall picture of the temperature-dependent interphase system is illustrated by the separation of β-carotene isomers performed with the C_{30} phase at various temperatures. At 285 K the all-*trans* and the 9-*cis* isomers are well separated (Figure 5). An increase in temperature leads to decreased retention times and selectivity factors. Nonetheless, a good separation is achieved at 295 K with shorter retention times. No severe changes occur up to a temperature of 305 K, but between 305 and 315 K the separation capability of the C_{30} phase decreases, and there is coelution of both stuctural isomers at 315 K.

Parallel to the decrease in separation quality of the C_{30} phase, the increase in temperature between 295 and 315 K leads to a change in the ratio of *trans* to *gauche* conformations of the *n*-alkyl chain. The fraction of *trans* conformations continuously decreases with increasing temperature. Under the separation conditions used at 295 K the fraction of *trans* conformations is 70%, and decreases to 57% at 305 K and finally to 35% at 315 K. Therefore, the increased specific selectivity for the sterical recognition of structural isomers is

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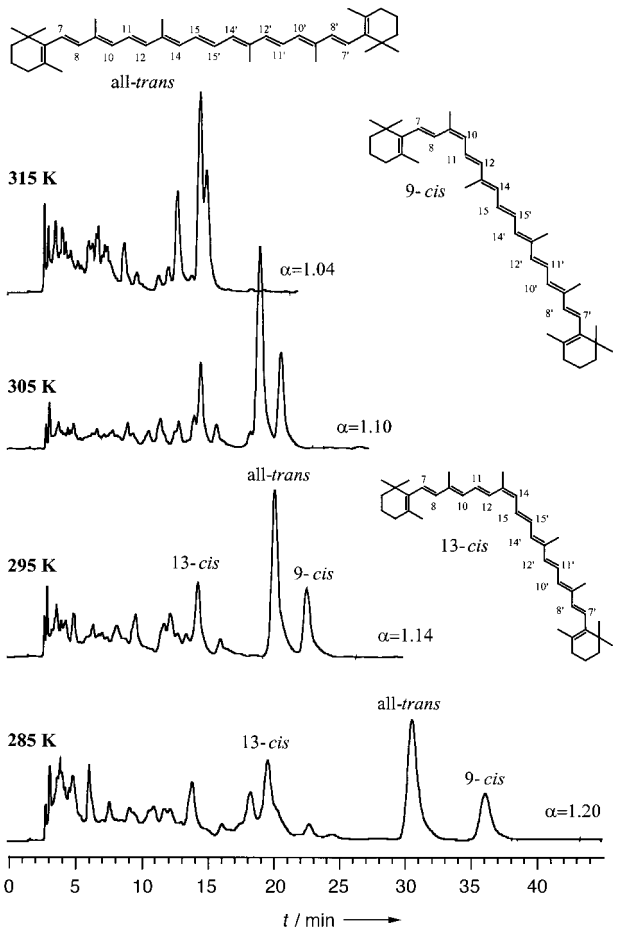

Figure 5. Separation of *cis* and *trans* isomers of β-carotene with a C_{30} phase at various temperatures. The indicated selectivities α refer to the relative retention behavior of the 9-*cis* and the all-*trans* isomers.

directed by the ratio of *trans* to *gauche* conformations of the C_{30} alkyl chains. A higher fraction of *trans* conformations improves the shape selectivity of the C_{30} phases. As far as practical applications are concerned, an important conclusion is that, in contrast to applications employing C_8 and C_{18} phases, temperature equilibration of the C_{30} column should be performed.

Experimental Section

The C_{30} interphase was synthesized by allowing silica gel (4 g, Prontosil, particle size 3 μm, pore size 200 Å, Bischoff, Leonberg, Germany) to react with trichloro-*n*-triacontylsilane (5 g, ABCR, Karlsruhe, Germany). The silane was dissolved in xylene (50 mL) at 343 K and decanted through a paper towel to silica gel suspended in xylene (50 mL). H_2O (1.0 mL) was added to start the reaction. After the mixture was heated at reflux for 10 h, the reaction product was isolated by filtration through a glass frit (P4) and washed with xylene, acetone, ethanol, H_2O, ethanol, acetone, and *n*-hexane.[1, 4]

NMR parameters: NMR spectra were recorded on a Bruker ASX300 spectrometer.^{13}C CP/MAS NMR measurements were performed with a 7-mm probe at a spinning rate (MAS) of 4000 Hz (pulse angle 90°, proton pulse length 6.5 μs, 2048 transients, contact time 6 ms, delay time 1 s, time domain 2K data points (TD) with a spectral width of (SW) 23 kHz, acquisition time 45 ms). Suspended-state NMR spectra were recorded with a 4-mm probe at a spinning rate of 3000 Hz (pulse angle 90°, pulse length 3.6 μs, 8000 transients, delay time 5 s, time domain 6K data points (TD) with a spectral width (SW) of 23 kHz, acquisition time 135 ms). All NMR measurements were performed after an equilibration time of 15 min.

2D WISE experiment: 650 transients per FID, time domain 2K data points (TD2) with a spectral width (SW2) of 23 kHz, acquisition time 45 ms, 64 increments (TD1) at 3 μs, spectral width (SW1) 333 kHz, contact time 500 μs, delay time 1 s, exponential multiplication with 20 Hz along F_2 and with 5000 Hz along F_1.

Fluorescence parameters: Stationary fluorescence anisotropies were determined in diluted suspensions (10^{-7} M solutions of DPH) on a Spex-222-Fluorolog spectrometer equipped with calcite polarizer prisms. The high dilution was necessary to prevent fluorescence depolarization by multiple scattering.

Chromatographic parameters: The separation material was added as a slurry in propan-2-ol to a stainless steel column (250 × 4.6 mm, Bischoff, Leonberg, Germany). The separation was carried out on a HP-1100 chromatographic system (Hewlett–Packard, Waldbronn, Germany) with methanol/TBME (75/25, v/v) as eluent (flow rate 1.0 mL min^{-1}, UV detection at 450 nm). An aliquot (10 μL) of a 0.05% solution of the β-carotene isomers was injected onto the column. The temperature of the HPLC separation was controlled by calibrating the column in a Haake K thermostat at the desired temperature.

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