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NMR Spectroscopy

DOI: 10.1002/anie.200601622

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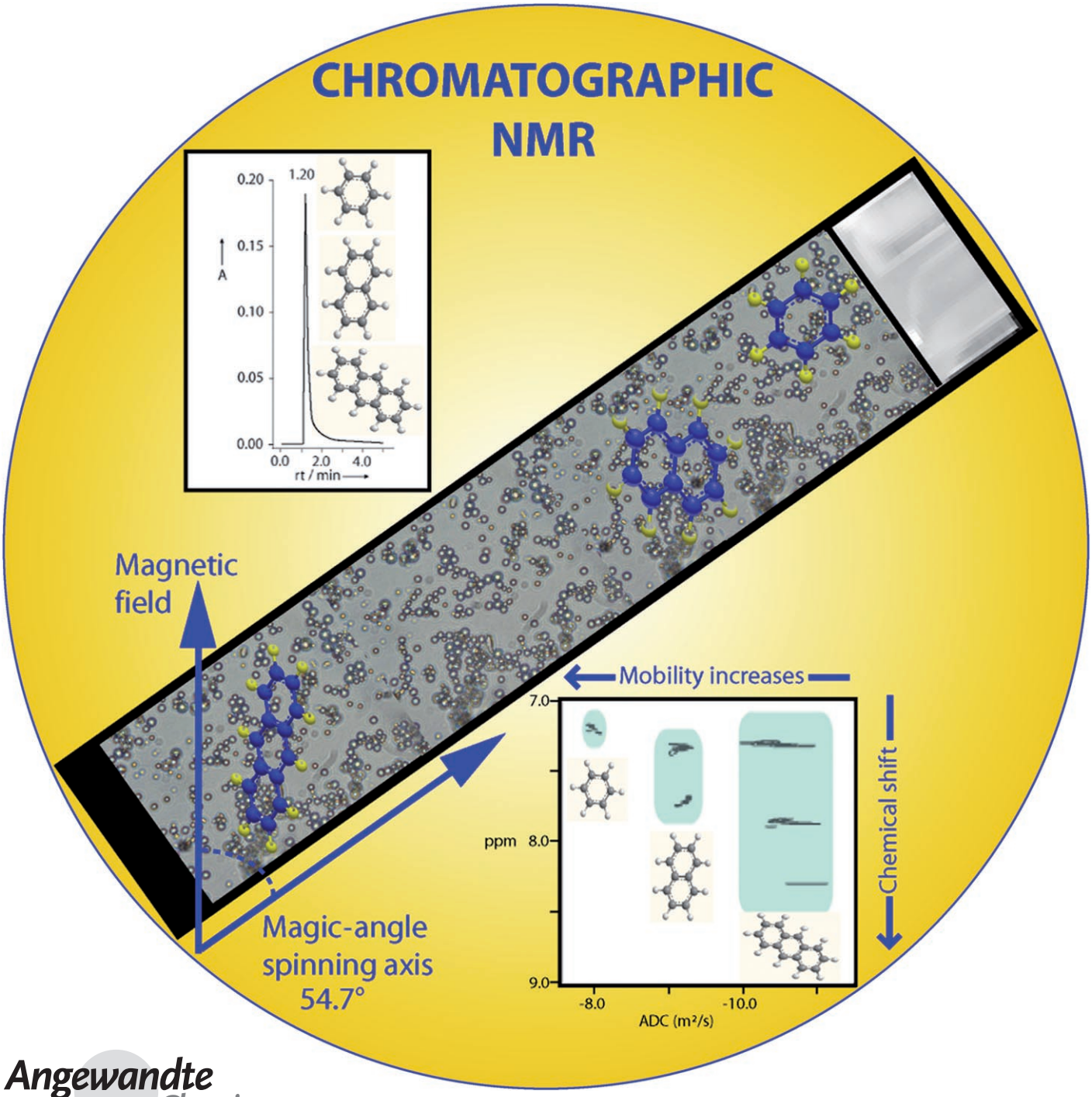

The determination of the components of assemblies of small molecules is an extremely common task in chemical laboratories, and HPLC is the preferred technique for separation and analysis of complex mixtures. A wide variety of sophisticated functionalized chromatographic solid supports has been developed (octadecylsilyl (ODS), cyano, amino) and adapted to different analytical problems. Recently, we introduced an alternative for the identification of the components in a mixture, without any chromatography involved, by using solid-enhanced diffusion-based NMR spectroscopy.[1,2] The method mimics a part of the chromatographic process in that the molecular average mobilities in the mixture are modified according to the individual affinities for the stationary phase. This enhances the spectral-separation capabilities of diffusion-based pulsed field gradient (PFG) experiments, such as DOSY.[3] Herein we show that, at least in favorable cases, this technique can provide a simpler approach to mixture analysis, yielding separation of the spectral components by using simple solid supports, such as regular porous silica gels.

A homologous series of aromatic compounds (benzene, naphthalene, and anthracene) was used as the test mixture. The usual procedure for chromatographic separation for this kind of compound requires reversed-phase (RP) partition chromatography, using ODS-functionalized silica and an organic/water mixture as the mobile phase (Figure 1a). Aromatic moieties are also known to interact with untreated silica gels because they are electron donor moieties and bare silica is an electron acceptor moiety.[4] However, on silica gel and using the same eluent conditions as for RPLC, namely an acetonitrile/water mixture, no solute separation is recorded on the chromatogram (Figure 1b). Direct-phase liquid chromatography also does not separate aromatic molecules.

Molecular diffusion coefficients can be measured by NMR spectroscopy by using PFG.[5–7] In fact, the molecular mobility has long been exploited in NMR spectroscopy as an independent dimension, as exemplified by the DOSY approach, which correlates the NMR spectrum on one dimension and the associated self-diffusion rates on a second dimension. The resulting layout is reminiscent of TLC. The enhanced performance of this application of high-resolution magic-angle spinning (HRMAS)–DOSY NMR spectroscopy[1] descends from the addition of a solid chromatographic support to the mixture. Selective affinity for the solids varies according to the molecular mobility and results in improvement in the resolution. Experiments were performed under HRMAS[8] conditions in which the ^{1}H NMR spectrum is recorded while spinning the sample oriented at the so-called magic angle with respect to the magnetic field. Relatively fast spinning rates are easily achieved by current technology and induces averaging of magnetic susceptibility broadening and other sources of decreased resolution of the spectrum.[9] Consequently, the NMR proton spectrum of the mixture thus obtained has a spectral quality close, although still reduced, to the one recorded without the solid phase. In Figure 2, we analyze the performance of this method in terms of separation of the spectral components. ODS-substituted silica (Figure 2a) provides a satisfactory separation of the peaks. An increase in the apparent-diffusion-coefficient (ADC) logarithm is equivalent to a decrease in the HPLC

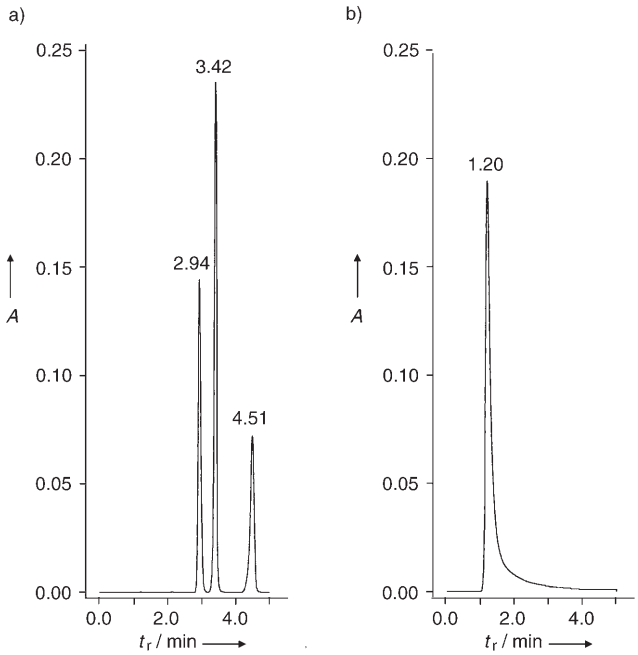

Figure 1. Aromatic homologous mixture chromatogram on a) an ODS column and b) a silica gel stationary phase. Eluent: acetonitrile/water, (90/10, v/v). Length of columns 250×4 mm. Flow rate 1.0 mL min^{-1}. Detection wavelength: 254 nm. t_r = retention time.

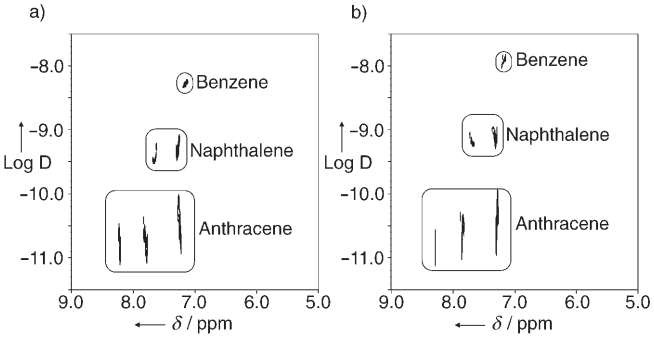

Figure 2. ^{1}H DOSY NMR spectra of an aromatic homologous series in acetonitrile/water (90/10, v/v) a) with an ODS chromatographic stationary phase and b) with a bare silica gel. D = apparent diffusion coefficient.

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[**] This study was supported in part by a grant from the French Conseil Régional Provence-Alpes-Côte-d'Azur and the Pharmaceutical Laboratory DistriB3 (Marseille, France). We thank VWR International France for providing all chromatographic stationary phases used herein.

retention time. The NMR spectroscopy retention scale obtained is therefore consistent with RPLC: benzene is "eluted" first then naphthalene and, finally, anthracene with a good resolution in both HPLC and NMR spectroscopy methods. The surprising outcome of a similar analysis performed by using bare silica gel (Figure 2b) is that a separative quality that is comparable to RP conditions is achieved. Indeed, about the same ADCs were measured and the diffusion resolution is in the same order of magnitude as with ODS chromatographic stationary phase, whereas in LC no separation was observed between these molecules on bare silica.

The NMR spectroscopy result is all the more surprising as the acetonitrile/water mixture is in competition with the aromatic molecules for adsorption on the silica gel. The differences between HPLC and the HRMAS–DOSY results must stem from the absence of flow and the higher solid-to-liquid ratio used in the NMR spectroscopy experiment. Experiments are underway to investigate further the mechanistic aspects.

This promising NMR spectroscopy method has some significant limits. The first is a technological one: only molecules that diffuse on timescales accessible with the available gradient strength (about 10^{-8} to 10^{-11} $m^2 s^{-1}$) on a conventional HRMAS probe can be observed. A first amelioration of this aspect can be achieved by using a special setup with a microimaging gradient system.[10] The necessity of good resolution in both the NMR and diffusion dimension is another limiting factor. Even under the beneficial effects of HRMAS, the line width remains broader than in solution-state NMR spectroscopy, and spectral overlap in frequency dimension may introduce artifacts in the processing. In fact, the diffusion-dimension layout is calculated by measuring an exponential decay[11] for which current day algorithms are still prone to misinterpret the results and so to produce a meaningless DOSY diagram. This blockage can surely be progressed upon as hinted by recent advances on the achievable precision of more-adapted processing methods for DOSY processing.[12,13] Moreover, it should be noted here that diffusion in the presence of porous solids may be more complex than the simple monoexponential model suggests as this behavior corresponds to a rather limited case.[14] This may partly explain the significantly worse resolution observed in our HRMAS-DOSY experiment when compared with the corresponding free liquid.

Although conceptually similar, chromatographic NMR spectroscopy and LC do not share all elementary steps, notably because of the absence of the flow of the mobile phase. The solid-induced deceleration of the tested molecules appears to be very similar in both bonded and bare silica gels. Consequently, good separation is achieved with very basic chromatographic materials, which may result in a simplification in the analysis of mixtures. As stated previously, a hint for the physical basis of this result could be provided by the affinity of aromatic molecules for silica gels. The full potential of the approach remains to be probed, but the illustration provided herein already points out a strong correspondence between chromatographic NMR spectroscopy and LC.

Experimental Section

The chromatographic phases used were Lichrospher100 Si (5 μm) and Lichrospher100 C18 (5 μm). For the NMR spectroscopy analysis, the test mixture was benzene (90.0 $g L^{-1}$), naphthalene (9.0 $g L^{-1}$), and anthracene (1.6 $g L^{-1}$) dissolved in CH_3CN/H_2O (90/10 v/v). The HRMAS rotor contained about 30 mg of chromatographic stationary phase and 10 μL of the test mixture. Roughly half of the stationary phase was introduced inside the rotor, then the solution was added and finally, the rotor was filled with the remaining part of the solid. All NMR spectroscopic experiments were performed at 400 MHz on a Bruker Avance spectrometer equipped with a ^1H HRMAS probe head producing magic-angle gradients with a maximum strength of 55 $G cm^{-1}$. Samples were prepared in 4 mm ZrO_2 rotors with a detection volume of 50 μL. The spectra were recorded at a spinning rate of 4000 Hz, at 303 K with a free induction decay containing 2 or 4 K data points. The pulse sequence used was based on the stimulated echo and incorporated bipolar gradient pulses and a longitudinal eddy current delay (BPP–LED).[15] The shape of all gradient pulses was sinusoidal. The gradients were generated through a built-in coil, which was aligned to the magic angle. No rotor synchronization was necessary. The spoil gradient pulses were 1 ms and the LED was 5 ms. The 2D diagram was realized with a gradient pulse of 2000 or 2200 μs and a diffusion time of 500 or 800 ms for the silica gel and for the bonded chromatographic stationary phase, respectively. A BPP–LED experiment was realized by using 16 different gradient values with longitudinal spacing, varying from 2 to 95% of the maximum gradient strength; for each of them 16 scans were recorded. For the processing, 128 points were represented in the diffusion dimension.

Chromatograms were recorded by using a L-6000 pump (Merck), a Jasco UV-975 detector, and a D-2500 integrator (Merck). The columns used were Lichrospher100 C18 250 × 4 mm (5 μm) and Lichrochart60 Si 250 × 4 mm (5 μm). The eluent was CH_3CN/H_2O (90/10 v/v) with a flow rate of 1.0 $mL min^{-1}$. The detection wavelength was 254 nm. The test mixture was benzene (770 $mg L^{-1}$), naphthalene (90 $mg L^{-1}$), and anthracene (5 $mg L^{-1}$) dissolved in the eluent.

Received: April 25, 2006
Revised: June 20, 2006
Published online: July 26, 2006

Keywords: analytical methods · chromatography · diffusion · magic-angle spinning · NMR spectroscopy

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