Journal of Chromatography A, 1257 (2012) 204–207

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Chromatographic-nuclear magnetic resonance can provide a prediction of high-pressure liquid chromatography shape selectivity tests

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ARTICLE INFO

Article history:
Received 18 April 2012
Received in revised form 9 June 2012
Accepted 10 July 2012
Available online 25 July 2012

Keywords:
PFG-NMR
Diffusion
RPLC
Retention model

ABSTRACT

NMR diffusometry has been recently demonstrated as a means of investigating the mobility variations of solutes induced by chromatographic phases (under the acronym chromatographic-NMR). Particularly, a given compound has its average diffusivity reduced proportionally to its affinity towards the solid. In this work we propose the first comparison of chromatographic-NMR and tests for assessment of column performance, to investigate to what measure the novel approach could provide an assay of the outcome of a given stationary phase without the need of packing the relative column. Specifically, using bulk materials, we reproduce with very good agreement a shape selectivity test as reported in the catalog of a column producer, consisting of four probe molecules, applied to two different stationary phases.

1. Introduction

NMR has often been associated to LC, for instance as a hyphenated detector [1] due to its remarkable reproducibility and ability at resolving the stereochemistry of small and heavy bio-organic compounds alike. Although this sequential application of chromatography and NMR spectroscopy for the characterization complex mixtures is an established analytical protocol, methods have been often sought to determine the composition of such complex samples purely spectroscopically [2], to minimize the sample manipulation. The NMR measurement of molecular mobilities or equivalently their diffusivities has a long tradition, including chromatography [3–5], and it is one of the methods that have been often proposed for the speciation of mixtures. Moreover, it is the NMR approach that resembles the closest LC (particularly in its most popular layout, Diffusion-Ordered Spectroscopy (DOSY) [6]), as molecules are differentiated effectively if their diffusivities are sufficiently different. To amplify such differences, a slowly moving or immobile capturing matrix can be added to the mixture [7–12]. Chromatographic-NMR (Chrom-NMR) is a recent version of matrix-enhanced NMR diffusometry, which relies on typical chromatographic solids [7,13–17]. Analytical versions of the method have been demonstrated using bare and functionalized silica [13–15,18,19] or polymers [20,21]. The addition of a solid causes loss of spectral resolution and may impose the use of specific NMR tools (e.g. High-Resolution Magic Angle Spinning (HRMAS), which has been used on several occasions to study phases/solutes interactions [22–32]), or by matching the susceptibility of the solvent and the solid (silica in this case) [18]. Silica-based Chrom-NMR was capable of outperforming its LC analog for an HILIC separation of aromatic homologs [15] and to perform spectral separation with mobile phases [17] not optimized for chromatography. Although these findings reinforced on one hand the analytical potential of the method, they also raised a number of questions on the thermodynamics of separation on silicas. Recently, the first step of a microscopic explanation of this specific physical-chemistry was provided, showing that differences in intraparticle and interparticle diffusion can be the reason for changes in the resolving power [33,34]. Particularly, the Chrom-NMR and LC discrimination power were shown to be comparable if the solution-to-solid phase ratio in the former technique was high, probably meaning that all intra- and interparticle space were accessed by the analytes [34]. With these premises, the conditions are now set to explore the capability of Chrom-NMR to reproduce, and thus predict LC. In this first exploratory study, we approach the classic case of shape selectivity tests in reverse phase LC using PAH molecular probes.

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http://dx.doi.org/10.1016/j.chroma.2012.07.054

2. Materials and methods

2.1. Samples

The bulk chromatographic phases were obtained from Dionex. All chemicals were purchased from Sigma–Aldrich and the deuterated solvents were obtained from Eurisotop (Saint-Aubin, France).

2.2. NMR measurements

All NMR experiments were performed at 400 MHz on a Bruker Avance (Wissembourg, France) spectrometer equipped with a ^{1}H HRMAS probe head capable of producing gradients along the magic angle axis, at a temperature of 303 K, at a spinning rate of 4000 Hz. For measurements in the presence of the solid phase, a solution 1 mg mL^{-1} in MeOD/D$_2$O 90/10 (v/v) was added to 15 mg of solid in a 4 mm zirconium rotor of 50 μL volume, while 12 μL rotors were used for the experiments on pure solutions [35].

HRMAS DOSY experiments were performed with the BPPLED pulse sequence. The gradient strength, G, was linearly incremented in 16 steps from 6 to 95% of its maximum value (62G cm^{-1}), with optimized PFG lengths, from 0.6 to 0.9 ms for pure solutions and from 1.1 to 2.2 ms in the presence of the phase, respectively for faster and slower average mobilities. The diffusion time Δ was 80 ms in all cases. The values of the apparent diffusion constant were obtained using the built-in fitting functions of the Bruker Topspin software (version 2.1), obtained as the average of the values corresponding to each peak of the molecule.

3. Theory

The analysis of diffusivities in porous materials by PFG-NMR spectra is well established [36,37]. Only the elements required for the interpretation of the presented results will be thus outlined in the following. A pulse of magnetic field gradient of intensity G, applied for a time δ, induces a position-dependent, continuous change of the signal phase along its direction of application. As a consequence, the overall signal becomes null. The application of a second PFG, of reversed intensity, produces a perfect compensation of the dephasing and thus the recovery of the signal intensity. If a molecule moves between the application of the first and second gradient pulse, the compensation will not occur, so that the larger the average displacement of a species, the weaker its recovered signal intensity. This is summarized by the following equation for the intensity of this echo experiment

$$I = I_0 \, \exp(-Dq^2(\Delta - \varepsilon)) \quad (1)$$

where $q = \gamma G\delta$, with γ the gyromagnetic ratio of the nucleus, I_0 is the signal intensity measured for a very small value of G, Δ between the PFG applications to allow diffusion and ε is a quantity that depends on the length and shape of the PFG. The coefficient D is typically estimated by running a series of experiments with variable G, which has the advantage of not having to take into account relaxation directly.

In complex media, where several different phases can coexist, the simpler case to treat is that of fast interphase equilibration (on the NMR and diffusion timescale), where D is an apparent diffusion coefficient [38,39]. If the multiphase system under study is that of a volatile solute partially adsorbed on a surface, it becomes [33]

$$D = x_b D_b + x_{ads} D_{ads} + x_V D_V \quad (2)$$

where x_b, x_{ads} and x_V are the molar fractions of bulk, adsorbed and vapor phase molecules, respectively and D_b, D_{ads} and D_V are the respective diffusion. For microporous materials that both the D_{ads} and D_V will have an impact for very low solution-to-solid phase ratios [33]. On the other hand, agreement between PFG-NMR chromatographic experiments and their LC counterparts is found when the porous volume is largely occupied [34]. Under these conditions relation (2) can be approximated as:

$$D \approx x_b D_b \quad (3)$$

and thus

$$x_b \approx \frac{D}{D_b} \quad (4)$$

This quantity represents the facility of a solute to evade the solid influence, so that its reciprocal D_b/D should be rather used for comparison with LC retention data. Finally, the D_b value can be related to the diffusion in pure solutions, D_0, as

$$D_b = D_0 \varepsilon \quad (5)$$

where ε is the porosity of the medium, that is to say the amount of the total volume available for the molecules to diffuse. In the case of our experiment, ε is not a straightforward parameter to calculate, but it is a constant for any material as it depends on the silica intraparticle porosity, the particle shape, the grafting and coverage. Thus, the parameter D_0/D will follow the same trend as D_b/D for a same phase and it will be used in its stead in the following. It remains to be understood in what cases the NMR measurements, with no flow of the mobile phase, can be expected to be representative of the LC analysis. One key point is the presence of fast exchange kinetics, which would imply that the thermodynamics will be ruling the chromatographic outcome, so that the absence of a flow can be neglected as a first approximation. The fast equilibrium approximation can be verified by inspection of the HRMAS NMR 1D spectra and PFG-NMR decays, characterized by the presence of a signal per molecule and a monoexponential decay for the trend depicted by Eq. (1). Slow exchange and intermediate kinetics produce intricate lineshapes required a more detailed modeling [38].

4. Results and discussion

To assess the capacity of Chrom-NMR to predict the result of the selectivity test over different chromatographic solids, two different column materials were used as solid supports: a standard C18 functionalized silica (Acclaim 120 – "A 120 C18" in the following) of 12 nm pore size and a polar embedded material, based on the same porous silica and containing a sulfonamide group (Acclaim PolarAdvantage – "A PA" in the following). A typical HRMAS spectrum is shown in Fig. 1, showing that the signals of the probe molecule (phenanthrene in this case) are well separated from the peaks originating from the grafted moieties or the solvents (and their sidebands).

Chrom-NMR is only capable of reproducing results consistent with LC if a high solution-to-solid phase ration was employed, of the order of 5–6 for bare silica [34]. The experimental conditions used here approximate well this situation. Deuterated solvents were used as they have been shown to provide, to first order, the same results as protonated ones [14,34]. Table 1 summarizes the measured apparent diffusion coefficients, for pure solution and in the presence of the two phases, for the four test molecules: toluene, phenanthrene, o-terphenyl and triphenylene. The planar polynuclear aromatic hydrocarbons (PAHs) are used to establish the linear dependence of the retention of the molecular size, the deviation from which of non-linear orthophenyl constitute the classic Tanaka's test. A more complete discussion on shape selectivity tests can be found in the literature. Note that these compounds have the additional advantage of presenting NMR signals in a region of the spectrum that is not overlapping with peaks originating from the phase, a property that simplifies and renders more accurate the analysis. To further increase the precision of the analysis, each